(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 February 2003 (06.02.2003)

PCT

(10) International Publication Number
WO 03/010202 A1

(51) International Patent Classification[7]: C07K 16/46

(21) International Application Number: PCT/KR02/01427

(22) International Filing Date: 26 July 2002 (26.07.2002)

(25) Filing Language: Korean

(26) Publication Language: English

(30) Priority Data:
2001-45028 26 July 2001 (26.07.2001) KR

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI,

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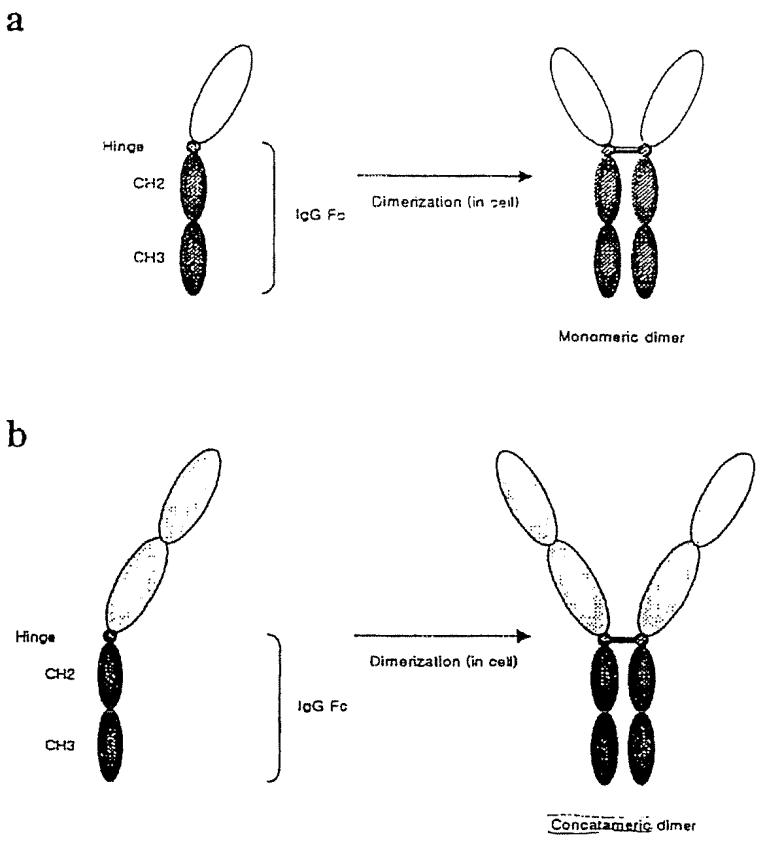
(54) Title: CONCATAMERIC IMMUNOADHESION



(57) Abstract: Disclosed are concatameric proteins comprising two soluble domains, in which the C-terminus of a soluble domain of a biologically active protein is linked to the N-terminus of an identical soluble domain or a distinct soluble domain of a biologically active protein. Also, the present invention disclosed dimeric proteins formed by formation of intermolecular disulfide bonds at the hinge region of two monomeric proteins formed by linkage of a concatamer of two identical soluble extracellular regions of proteins involving immune response to an Fc fragment of an immunoglobulin molecule, their glycosylated proteins, DNA constructs encoding the monomeric proteins, recombinant expression plasmids containing the DNA constructs, host cells transformed or transfected with the recombinant expression plasmids, and a method of preparing the dimeric proteins by culturing the host cells. Further, the present invention disclosed pharmaceutical or diagnostic compositions comprising the dimeric protein or its glycosylated form.

WO 03/010202 A1

SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) **Designated States** *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

CONCATAMERIC IMMUNOADHESION

TECHNICAL FIELD

The present invention relates to concatameric proteins, and more specifically, concatamerized structure of biologically active protein domains where C-terminal end of extracellular soluble domain of biologically active protein is fused to N-terminal end of the same or other extracellular soluble domain of biologically active protein, and dimerization of two concatamers by coupling to hinge region of Fc fragment of immunoglobulin, and glycosylated forms of the concatameric proteins.

BACKGROUND ART

The activity of cytokine is associated with pathologic severity of inflammatory and /or immune response to various antigenic stimulations. Many antigen specific antibodies and soluble receptors which could recognize cytokines are currently in use to inhibit the function of cytokines for the therapeutic purposes (WO 93/016184, WO 96/02576, WO 96/023067, WO 1997/03682, and US 5,434,131, 5,656,272, 5,977,318, 6,210,661, 6,225,117). Antibodies and soluble receptors inhibit cytokine signal transduction by disturbing interaction between cytokines and their receptors on cell surface.

Soluble receptors used as functional inhibitors of cytokine that fused to heavy chains of human immunoglobulins were disclosed by Capon et al. (Nature 337:5254, 1989), and thereafter many patents were disclosed inventions related to fusion proteins of soluble receptors and immunoglobulins (US patent 5,521,288, 5,844,095, 6,046,310, 6,090,914, 6,100,383, 6,225,448).

Generally, fusion proteins of soluble receptors and immunoglobulins have following advantages (Capon et al., Nature 337:5254, 1989)

1. Increase in total avidity to ligand by forming bivalency via dimerization.
2. Increase in blood half-life of proteins, that is, increase in molecular stability
3. Activation of effecter cells by Fc fragment of immunoglobulin heavy chain
4. Convenience of purification by using affinity column, e.g. using protein A

Most fusion proteins of receptor extracellular domain and immunoglobulin heavy chain are composed of heavy chain without CH1 domain, which result in dimers not binding to light chains. This structure is more desirable for the function of proteins and receptors involving immune response. For example, TNFR(WO92/16221, WO95/34326)-immunoglobulin fusion proteins disclosed in WO94/06476 and US 5,447,851 have been used for the inhibition of TNF-mediated inflammation. It is well known that TNFR-immunoglobulin fusion proteins have a higher affinity than original monomeric molecules (Lesslauer et al., Eur. J. Immunol. 21:2883, 1991; Ashkenazi et al., Proc. Natl. Acad. Sci. 88:10535, 1991; Peppe et al., J. Exp. Med. 174:1483, 1991; Mohler et al., J. Immunol. 151:1548, 1993).

For the improved inhibition of TNF mediated response, one can increase efficacy by multimerizing soluble extracellular domains of TNFR, CD2, and CTLA-4. For example, when fusion proteins of TNFR's extracellular domains bound with immunoglobulin heavy chain(heavy chain fusion protein) and with light chain(light chain fusion protein) respectively are coexpressed in the same cell, one can produce fusion proteins as a tetrameric form by linking heavy chain to heavy and light chains. This tetramer showed much more increased efficacy than monomeric or dimeric forms as presented by Scallon et al. (Cytokine 7:759, 1995).

However, this method had many difficulties for commercialization such as simultaneous expression of two different fusion genes in the same cell line, remarkably lower production yields of multimeric form; and difficulty in purifying multimeric high

molecular weight forms. For these reasons, immunoglobulin fusion proteins currently in use are only heavy chain fused form.

Therefore, there is considerable demand for the development of methods of producing multimeric protein therapeutics with high yield and efficient purification procedures.

DISCLOSURE OF INVENTION

The present inventors have manufactured concatameric proteins by fusing the C-terminal end of soluble domain of biologically active protein to the N-terminal end of soluble domain of the same or other biologically active protein by using DNA recombination techniques. Also, the present inventors have dimerized this concatamers by linking it to the hinge region of Fc fragment of immunoglobulin and added more glycosylations by using DNA mutagenesis techniques. And the present inventors have found that concatamerized protein dimers and their glycosylated forms show increased efficacy and stability compared to conventional monomeric fusion proteins.

Therefore, in one aspect, the present invention provides concatameric proteins where C-terminal end of soluble domain of biologically active proteins is fused to N-terminal end of soluble domain of the same or other biologically active proteins.

In another aspect, the present invention provides dimeric proteins formed by disulfide bond at hinge region of two monomeric proteins whose concatamerized part is fused to hinge region of Fc fragment of immunoglobulin.

Also in another aspect, the present invention provides DNA constructs that encode monomeric fusion proteins whose concatamerized domain is fused to hinge region of Fc fragment of immunoglobulins.

Also in another aspect, the present invention provides DNA plasmids comprising a DNA construct that encodes monomeric fusion protein whose concatamerized part is fused to hinge region of Fc fragment of immunoglobulin.

Also in another aspect, the present invention provides host cells transfected or transformed with recombinant DNA plasmids including a DNA construct that encodes monomeric fusion protein whose concatamerized part is fused to hinge region of Fc fragment of immunoglobulin.

Also in another aspect, the present invention provides a method for culturing the host cells, which were transfected or transformed with recombinant DNA plasmids including a DNA construct that encodes monomeric fusion protein whose concatamerized part is fused to hinge region of Fc fragment of immunoglobulin, under culture condition for expression of DNA constructs encoding concatameric fusion protein coupled to hinge region of Fc fragment of immunoglobulin , and manufacturing dimeric concatamers formed by disulfide bond at hinge region of two monomeric concatamers described as above including the process of purification of the proteins described as above from cell culture.

Also in another aspect, the present invention provides a method for culturing the host cells, which were transfected or transformed with recombinant DNA plasmids including a DNA construct that encodes monomeric fusion protein whose concatamerized part of immunomudulatory function is fused to hinge region of Fc fragment of immunoglobulin and is inserted with glycosylation motifs, under the best condition which is suitable for expression of DNA constructs that encode monomeric fusion protein whose concatamerized part of immune function is fused to hinge region of Fc fragment of immunoglobulin, and for manufacturing glycosylated dimers formed by disulfide bond at hinge region of two monomeric proteins described as above including the process of purification of the glycosylated proteins described as above from cell culture.

Also in another aspect, the present invention provides DNA primers for inserting glycosylation motif into the DNA constructs that encode monomeric fusion

proteins whose concatamerized part is fused to hinge region of Fc fragment of immunoglobulins.

Also in another aspect, the present invention provides the glycosylated dimers formed by disulfide bond at hinge region of two monomeric proteins whose concatamerized part involving immune response is fused to hinge region of Fc fragment of immunoglobulins.

Also in another aspect, the present invention provides the pharmaceutical compositions comprising dimers formed by disulfide bond at hinge region of two monomeric proteins whose concatamerized part involving immune response is fused to hinge region of Fc fragment of immunoglobulins in a pharmaceutically effective amount and in a pharmaceutically acceptable carrier.

Also in another aspect, the present invention provides the pharmaceutical compositions comprising glycosylated dimers formed by disulfide bond at hinge region of two monomeric proteins whose concatamerized part involving immune response is fused to hinge region of Fc fragment of immunoglobulins in a pharmaceutically effective amount and in a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

Fig. 1 is a schematic view showing a process of preparing a DNA construct encoding a conventional simple fusion monomeric protein through polymerase chain reaction (PCR);

Fig. 2 is a schematic view showing a process of preparing a DNA construct encoding a concatameric fusion monomeric protein according to the present invention through PCR;

Fig. 3a shows structures of $[TNFR/Fc]_2$, $[CD2/Fc]_2$ or $[CTLA4/Fc]_2$ fusion proteins, which are simple fusion dimeric proteins formed through homodimerization in cells of TNFR/Fc, CD2/Fc or CTLA4/Fc fusion proteins as examples of conventional simple fusion monomeric proteins;

Fig. 3b shows structures of $[TNFR-TNFR/Fc]_2$, $[CD2-CD2/Fc]_2$ or $[CTLA4-CTLA4/Fc]_2$ fusion proteins, which are concatameric fusion dimeric proteins formed through homodimerization in cells of TNFR-TNFR/Fc, CD2-CD2/Fc or CTLA4-CTLA4/Fc fusion proteins as embodiments of the concatameric fusion dimeric protein according to the present invention;

Fig. 4a shows a structure of $[TNFR1-TNFR1/Fc]_2$, as an embodiment of a concatameric fusion dimeric protein according to the present invention;

Fig. 4b shows a structure of $[TNFR2-TNFR2/Fc]_2$, as another embodiment of the concatameric fusion dimeric protein according to the present invention;

Fig. 4c shows a structure of $[CD2-CD2/Fc]_2$, as a further embodiment of the concatameric fusion dimeric protein according to the present invention;

Fig. 4d shows a structure of $[CTLA4-CTLA4/Fc]_2$, as a still further embodiment of the concatameric fusion dimeric protein according to the present invention;

Fig. 5 is a diagram showing a process of constructing a recombinant expression plasmid pTR11Ig-Top10′ expressing a concatameric fusion monomeric protein TNFR1-TNFR1/Fc according to the present invention;

Fig. 6 is a diagram showing a process of constructing a recombinant expression plasmid pCD22Ig expressing a concatameric fusion monomeric protein CD2-CD2/Fc according to the present invention;

Fig. 7 is a map of a recombinant expression plasmid pTR11Ig-Top10′ expressing a concatameric fusion monomeric protein TNFR1-TNFR1/Fc according to the present invention;

Fig. 8 is a map of a recombinant expression plasmid pTR22Ig-Top10′ expressing a concatameric fusion monomeric protein TNFR1-TNFR1/Fc according to the present invention;

Fig. 9 is a map of a recombinant expression plasmid pCD22Ig expressing a concatameric fusion monomeric protein CD2-CD2/Fc according to the present invention;

Fig. 10 is a map of a recombinant expression plasmid pCT44Ig expressing a concatameric fusion monomeric protein CTLA4-CTLA4/Fc according to the present invention;

Fig. 11 is a map of a recombinant expression plasmid pTR11Ig-MG expressing a concatameric fusion monomeric protein mgTNFR1-TNFR1/Fc containing four glycosylation motif peptides according to the present invention;

Fig. 12 is a map of a recombinant expression plasmid pTR22Ig-MG expressing a concatameric fusion monomeric protein mgTNFR2-TNFR2/Fc containing two glycosylation motif peptides according to the present invention;

Fig. 13 is a map of a recombinant expression plasmid pCD22Ig-MG expressing a concatameric fusion monomeric protein mgCD2-CD2/Fc containing two glycosylation motif peptides according to the present invention;

Fig. 14 is a map of a recombinant expression plasmid pCT44Ig-MG expressing a concatameric fusion monomeric protein mgCTLA4-CTLA4/Fc containing three glycosylation motif peptides according to the present invention;

Fig. 15 shows a result of SDS-PAGE of purified concatameric fusion dimeric proteins [TNFR1-TNFR1/Fc]$_2$ and [TNFR2-TNFR2/Fc]$_2$ under reducing or non-reducing conditions;

Fig. 16 is a graph showing inhibitory effect of the conventional simple fusion dimeric proteins [TNFR1/Fc]$_2$(●) and [TNFR2/Fc]$_2$(○) and the concatameric fusion dimeric proteins [TNFR1-RNFR1/Fc]$_2$(▼) and [TNFR2-TR2Fc]$_2$(▽) according to the present invention against cytotoxic activity of TNF-alpha;

Fig. 17 is a graph showing inhibitory effect of the conventional simple fusion dimeric proteins [TNFR1/Fc]$_2$(●) and [TNFR2/Fc]$_2$(○) and the concatameric fusion dimeric proteins [TNFR1-RNFR1/Fc]$_2$(▼) and [TNFR2-TR2Fc]$_2$(▽) according to the present invention against cytotoxic activity of TNF-beta;

Fig. 18 is a graph showing inhibitory effect of the conventional simple fusion dimeric protein [CD2/Fc]$_2$(●), the known immunosuppressive agent cyclosporin A (▼) and the concatameric fusion dimeric protein [CD2-CD2/Fc]$_2$(○) according to the present invention on the proliferation of active T lymphocytes;

Fig. 19 is a graph showing inhibitory effect of the conventional simple fusion

dimeric protein $[CTLA4/Fc]_2$(●), the known immunosuppressive agent cyclosporin A (▼) and the concatameric fusion dimeric protein [CTLA4- CTLA4/Fc]$_2$ (○) according to the present invention on the proliferation of active T lymphocytes;

Fig. 20 is a graph showing blood half-life of the conventional simple fusion dimeric protein $[TNFR1/Fc]_2$(●), the concatameric dimeric protein [TNFR1-TNFR1/Fc]$_2$ (○) and a glycosylated concatameric fusion dimeric protein [mgTNFR1-TNFR1/Fc]$_2$ (▽) according to the present invention;

Fig. 21 is a graph showing blood half-life of the conventional simple fusion dimeric protein $[CD2/Fc]_2$(●), the concatameric fusion dimeric protein [CD2-CD2/Fc]$_2$ (○) and a glycosylated concatameric fusion dimeric protein [mgCD2-CD2/Fc]$_2$ (▽) according to the present invention;

Fig. 22 is a graph showing blood half-life of the conventional simple fusion dimeric protein $[CTLA4/Fc]_2$(●), the concatameric fusion dimeric protein [CTLA4-CTLA4/Fc]$_2$ (○) and a glycosylated concatameric fusion dimeric protein [mgCTLA4-CTLA4/Fc]$_2$ (▽) according to the present invention; and

Fig. 23 is a graph showing inhibitory effect of PBS (●) as a control, the conventional simple fusion dimeric proteins $[TNFR1/Fc]_2$(■) and $[TNFR2/Fc]_2$(▲), and concatameric fusion dimeric proteins [TNFR1-TNFR1/Fc]$_2$ (×) and [TNFR2-TNFR2/Fc]$_2$ (△) according to the present invention on the induction of collagen-induced arthritis (CIA) in DBA/1 mice.

BEST MODE FOR CARRYING OUT THE INVENTION

The present invention is generally directed to concatameric proteins, and more particularly, to immunoadhesion molecules. Immunoadhesion molecules are typically formed by fusion of the Fc fragment of immunoglobulin (Ig) to a ligand-binding region of a receptor or an adhesion molecule, and thus have a structure similar to that of an antibody. The typical immunoadhesion molecules known in the art have a structure of an antibody in which the variable region is substituted with a ligand-binding region of a receptor while retaining the Fc fragment. A wide variety of immunoadhesion molecules are suggested in the literature. However, immunoadhesion molecules according to the

present invention have different structure with the conventional immunoadhesion molecules, and there is also no prior art predicting or describing preparation of the immunoadhesion molecules according to the present invention.

Definition of Terms

For full understanding of the characteristic structure of the immunoadhesion molecules according to the present invention, exact definitions of the terms used in the present invention are given as follows. In general, all of the technical and scientific terms being not additionally defined in the present invention have meanings commonly used in the art. However, although having meanings commonly used in the art, the following terms are defined to give a clearer understanding of their meanings and make the scope of the present invention clear, as follows.

The term "immunoglobulin", as used herein, refers to protein molecules being produced in B cells and serving as antigen receptors specifically recognizing a wide variety of antigens. The molecules have a Y-shaped structure consisting of two identical light chains (L chains) and two identical heavy chains (H chains), in which the four chains are held together by a number of disulfide bonds, including the disulfide bridge between the H chains at the hinge region. The L and H chains comprise variable and constant regions. The L chain variable region associates with the H chain variable region, thus producing two identical antigen-binding regions. According to features of the constant regions of H chains, immunoglobulins (Ig) are classified into five isotypes, A (IgA), D (IgD), E (IgE), G (IgG) and M (IgM). Each subtype possesses unique structural and biological properties. For example, IgG has slightly different Fc structure, compared with other isotypes. In addition, IgG and IgA have a number of subtypes. For example, the human IgG isotype has four subtypes, IgG1, IgG2, IgG3 and IgG4, which have $\gamma 1$, $\gamma 2$, $\gamma 3$ and $\gamma 4$ H chains, respectively. Biological functions of immunoglobulin molecules, such as complement activation, Fc receptor-mediated phagocytosis and antigen-dependent cytotoxicity, are mediated by structural determinants (complementarity-determining regions) in the Fc region of H chains. Such an Fc region of H chains is used for construction of dimeric proteins according to the present

invention, and may be derived from all isotypes and subtypes of immunoglobulin as described above.

The term "Fc fragment of an immunoglobulin molecule", as used herein, refers to a fragment having no antigen-binding activity and being easily crystallized, which comprises a hinge region and CH2 and CH3 domains, and a portion responsible for binding of an antibody to effector materials and cells. Therefore, the Fc fragment mentioned in the present invention can be different from that described in some literatures, but includes the hinge region. Such description of the Fc fragment is given to supply convenience in describing the present invention, and will be fully understood by those of ordinary skill in the art with reference to the specification of the present invention and the accompanying drawings.

The term "biologically active protein", as used herein, refers to a protein, peptide or polypeptide having generally physiological or pharmaceutical activities, which retains a part of its native activities after forming a concatamer or immunoadhesion molecule. The term "biological activity", as used herein, is not limited in meaning to physiological or pharmaceutical activities. For example, some concatamers, such as those containing an enzyme can catalyze a reaction in an organic solvent. Similarly, some high-molecular weight fusion molecules containing concanavalin A or an immunoglobulin molecule are useful as diagnostic agents in laboratories.

Non-limiting examples of the protein, peptide or polypeptide include hemoglobin, serum proteins (e.g., blood factors including factor VII, VIII and factor IX), immunoglobulin, cytokines (e.g., interleukin), α-, β- and γ-interferon, colony-stimulating agent (e.g., G-CSF and GM-CSF), platelet-derived growth factor (PDGF), and phospholipase activating proteins (PLAPs). Other typical biological or therapeutic proteins include insulin, plant proteins (e.g., lectin and ricin), tumor necrosis factor (TNF) and its related alleles, growth factors (e.g., tissue growth factors and endothelial growth factors such as TGFα or TGFβ), hormones (e.g., follicle-stimulating hormone, thyroid-stimulating hormone, antidiuretic hormone, pigment-concentrating or dispersing hormones and parathyroid hormone, luteinizing hormone-releasing hormone and its derivatives, calcitonin, calcitonin gene related peptide (CGRP), synthetic enkephalin, somatomedin, erythropoietin, hypothalamus releasing factors, prolactin, chronic gonadotrophin, tissue

plasminogen-activating agents, growth hormone-releasing peptide (GHRP), and thymic humoral factor (THF). The immunoglobulins include IgG, IgE, IgM, IgA, IgD and fragments thereof. Some proteins such as interleukin, interferon or colony-stimulating factor may be produced in a non-glycosylated form using DNA recombinant techniques. The non-glycosylated proteins may be useful as biologically active materials in the present invention.

In addition, the biologically active materials useful in the present invention include any polypeptide, which has bioactivity in vivo. Examples of the biologically active materials include peptides or polypeptides, fragments of an antibody, single chain-binding proteins (see U.S. Pat. No. 4,946,778), binding molecules including fusion polypeptides of antibodies or their fragments, polyclonal antibodies, monoclonal antibodies, and catalytic antibodies. Other examples of the biologically active materials include allergen proteins, such as ragweed, antigen E, honeybee venom, or allergen of mites.

In addition, the biologically active material useful in the present invention includes enzymes. Examples of the enzymes include carbohydrate-specific enzymes, proteinases, oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases. In detail, non-limiting examples of the enzymes include asparaginase, arginase, arginine deaminase, adenosine deaminase, peroxide dismutase, endotoxinase, catalase, chymotrypsin, lipase, uricase, adenosine dephosphatase, tyrosinase, and bilirubin oxidase. Examples of the carbohydrate-specific enzymes include glucose oxidase, glucodase, galactosidase, glucocerebrosidase, and glucouronidase.

The term "proteins involving immune response", as used herein, refers to all proteins mediating cell-to-cell signal transduction during cellular or humoral immune response and thus activating or suppressing immune response. Immunity is a process of protecting "self" from "non-self" such as bacteria or viruses. Immune response is largely divided into cellular and humoral immune response, where T and B lymphocytes play the most important role. T cells, mainly mediating cellular immune response, directly attack and kill virus-infected cells or tumor cells, or help other immune cells by secreting cytokines functioning to induce or activate immune response or inflammation. B cells produce antibodies against non-self foreign materials (antigens) that enter a body,

such as bacteria or viruses, and such immune response is called cellular immune response. Cell-to-cell signal transduction is an essential process in both cellular and humoral immune responses, in which a signal molecule, that is, a ligand, interacts with a cell surface receptor acting to transduce a specific signal into a cell.

Representative examples of the proteins involving the immune response according to the present invention include cytokines, cytokine receptors, adhesion molecules, tumor necrosis factor receptor (TNFR), enzymes, receptor tyrosine kinases, chemokine receptors, other cell surface proteins, and soluble ligands. Non-limiting examples of the cytokines include IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-17, TNF, TGF, IFN, GM-CSF, G-CSF, EPO, TPO, and M-CSF. Examples of the cytokine receptors, but are not limited to, include growth hormone receptors (GHRs), IL-13R, IL-1R, IL-2R, IL-3R, IL-4R, IL-5R, IL-6R, IL-7R, IL-9R, IL-15R, TNFR, TGFR, IFNR (e.g., IFN-γ R α-chain and IFN-γ R β-chain), interferon-α R, -β R and -γ R, GM-CSFR, G-CSFR, EPOR, cMpl, gp130, and Fas (Apo 1). Non-limiting examples of the enzymes include influenza C hemaglutinin esterase and urokinase. The chemokine receptors are exemplified by CCR1 and CXCR1-4. Examples of the receptor tyrosine kinases, but are not limited to, include TrkA, TrkB, TrkC, Htk, REK7, Rse/Tyro-3, hepatocyte growth factor R, platelet-derived growth factor R, and Flt-1. Examples of other cell surface proteins includes CD2, CD4, CD5, CD6, CD22, CD27, CD28, CD30, CD31, CD40, CD44, CD100, CD137, CD150, LAG-3, B7, B61, β-neurexin, CTLA-4, ICOS, ICAM-1, complement R-2 (CD21), IgER, lysosomal membrane gp-1, α2-microglobulin receptor-related proteins, and sodium-releasing peptide R. Non-limiting examples of the soluble ligands include IL-10, heregulin, and keratinocyte growth factors.

Ligands for the proteins involving immune response according to the present invention and use thereof are well known to those of ordinary skill in the art, as summarized in Tables 1 to 7, below.

TABLE 1

Proteins involving immune response: Adhesion molecules

Adhesion molecules	Ligands	Uses
CD4	HIV gp120	Inhibition of in vivo HIV infection; and identification of CD4 domain participating in ligand binding
L-Selectin	GlyCAM-1, CD34	Prevention of neutrophile-mediated lung damage; determination of position in tissues of a ligand by histochemical staining; and isolation and cloning of ligands and determination of their properties
E-Selectin	Sialyl Lewis^X	Prevention of neutrophile-mediated lung damage; and determination of thermodynamic properties in ligand-binding
P-Selectin	Sialyl Lewis^X	Prevention of neutrophile-mediated lung damage; and study of functions of individual of amino acid residues in binding to cell surface
ICAM-1	CD11a/CD18	Phagocytosis of erythrocytes in malaria; inhibition of infection with rhinovirus; and anti-inflammation in diabetes
ICAM-2	CD11a/CD18	Study of activation of T cells mediated by T cell receptor
ICAM-3	CD11a/CD18	Identification of receptor domains binding to a ligand
VCAM-1	VLA-4	Study of role of VLA-4 in T lymphocyte migration to dermal inflammation sites
LFA-3	CD2	Study of role of CD2 in costimulation of T cells
L1 glycoprotein	Fibroblast growth factor receptor	Stimulation of nerve reproduction after repair; and functional comparison with FGF

TABLE 2

Proteins involving immune response: Enzymes

Enzymes	Ligands	Uses
Influenza C hemaglutinin esterase	9-0-acetylated sialic acid	Inactive enzyme used in study of tissue-specific expression of ligands
Urokinase	Urokinase receptor	Inactive enzyme developed to inhibit cancer metastasis by disturbing urokinase activation

TABLE 3

Proteins involving immune response: Cytokine receptors

Cytokine receptors	Ligands	Uses
IFN-γ R α-chain	IFN-γ	Inhibition of IFN-mediated autoimmunity
IFN-γ R β-chain	IFN-γ	Study of structure of subunits of a ligand-receptor complex
IL1R	IL-1	Inhibition of IL-1-mediated inflammation
IL4R	IL-4	Identification of receptor domains participating in ligand binding
Erythropoietin R	Erythropoietin	Map design of epitopes of anti-ligand antibodies
cMpl	Thrombopoietin	Isolation and cloning of ligands
gp130	IL-6-IL6R complex	Study of structure of subunits of a ligand-receptor complex

TABLE 4

Proteins involving immune response: Tumor necrosis factor receptors

TNF receptors	Ligands	Uses
TNF R-1	TNF, lymphotoxin-α	Treatment of septic shock, rheumatoid arthritis and other inflammatory diseases; and identification of domains participating in ligand binding
TNF R-2	TNF, lymphotoxin-α	Inhibition of TNF-enriched HIV replication; and prevention of collagen-induced arthritis in mice
Lymphotoxin-β R	Lymphotoxin-β	Study of structure of subunits of cell surface lymphotoxin-β
Fas/Apo-1/CD95	Fas/Apo-1/CD95 ligand	Treatment of excessive apoptosis and related diseases (e.g., AIDS); and resistance to apoptosis of lymphocytes and peripheral immune tolerance; roles of Fas ligand in T cell-mediated cytotoxicity; and isolation and cloning of ligands
CD27	CD27 ligand	Isolation and cloning of ligands
CD30	CD30 ligand	Isolation and cloning of ligands
CD40	gp39	Isolation and cloning of ligands
4-1BB	4-1BB ligand	Identification of tissues containing ligands by histochemical staining; isolation and cloning of ligands; and Study of structural determinant of potential ligand
OX40	gp34	Isolation and cloning of ligands

TABLE 5

Proteins involving immune response: Receptor tyrosine kinases

Receptor tyrosine kinases	Ligands	Uses
TrkA, B, C	Neutropin	Determination of properties of neutropin binding
Htk	Htk ligand	Isolation and cloning of ligands
REK7	AL-1	Isolation and cloning of ligands
Rse/Tyro-3	Protein S, Gas6	Identification of ligands and determination of their properties
Hepatocyte growth factor R	Hepatocyte growth factor	Identification of receptor domains participating in ligand binding
Platelet-derived growth factor R	Platelet-derived growth factor	Identification of receptor domains participating in ligand binding
Flt-1	Vesicular endothelial growth factor (VEGF)	Determination of properties of ligand binding of receptors
Flk-1/KDR	VEGF	Evaluation of selectivity of receptors for VEGF versus placenta growth factor

TABLE 6

Proteins involving immune response: Other cell surface proteins

Other cell surface proteins	Ligands	Uses
B7	CD28	Study of T cell stimulation by B cells
B61	Eck	Roles of Eck in inflammation
β-neurexin	β-neurexin ligand	Determination of properties of a signal sequence from β-neurexin
CD2	LFA-3, CD48	Identification of ligands
CD5	CD5 ligand	Study of T cell stimulation by B cells
CD6	ALCAM	Study of binding activities of cloned ligands
CD22	CD45, other sialoglycoproteins	Identificaiotn of ligands; study on roles of CD22 in T-B-cell interaction; and determination of properties of binding determinants of sialo-oligo sugar ligands
CD28	B7, B7-2	Study of T cell stimulation by B cells
CD31	CD31	Identification of CD31 domains related to homotype binding
CD44	Hyaluronate	Screening of tissues containing ligands by histochemical staining; and determination of properties of structural determinants of ligands
Complement R-2 (CD21)	C3 fragment	Inhibition of reactivity of antibody to immunosuppressive and cancer therapeutic agents
CTLA-4	B7	Identification of CTLA-4 as a secondary receptor of B7
IgE R	IgE	Inhibition of mast cell-binding of IgE as therapy of allergic diseases
Lisosome membrane gp-1	LAMP-1 ligand	Design of epitope maps of anti-ligand antibodies
α2-microglobulin receptor-bound proteins	gp330	Determination of position of ligands in tissues by histochemical staining
Sodium-releasing peptide R	Sodium-releasing peptide	Design of epitope maps of anti-ligand antibodies; and preparation of recombinant receptors for structural study

TABLE 7

Proteins involving immune response: Soluble ligands

Soluble ligands	Ligands	Uses
IL-2	IL-2R	Extension of half-life of IL-2 in the circulation system
IL-10	IL-10R	Therapy of septic shock and transplantation rejection; and extension of half-life of IL-10 in the circulation system
Heregulin	Her4/p180^{erbB4}	Study of signal transduction by Her4
Keratinocyte growth factor	Keratinocyte growth factor R	Determination of position of receptors by histochemical staining

The term "soluble extracellular domain", as used herein, refers to a portion exposed to the extracellular region of an integral membrane protein penetrating the cell membrane comprising phospholipid, wherein the integral membrane protein contains one or more transmembrane domain made up predominantly of hydrophobic amino acids.

Such an extracellular domain mainly comprises hydrophilic amino acids, which are typically positioned at the surface of a folded structure of a protein, and thus is soluble in an aqueous environment. For most cell surface receptor proteins, extracellular domains serve to bind specific ligands, while intracellular domains play an important role in signal transduction.

The term "concatamer-linked", as used herein, refers to a state in which two soluble domains of biologically active proteins are linked and thus form a long polypeptide.

The term "concatameric protein", as used herein, means a concatamer-linked protein. For example, the N-terminus of a soluble extracellular domain of a protein involving immune response is linked to the C-terminus of an identical soluble extracellular domain of the protein involving immune response, wherein the C-terminus of the former soluble extracellular domain is linked to the hinge region of an Fc fragment of an immunoglobulin molecule. Thus, two identical soluble extracellular domains of a protein involving immune response form a long polypeptide.

The term "simple fusion monomeric protein", as used herein, refers to a fusion protein having a monomeric structure consisting of a single polypeptide formed by linkage of a soluble extracellular domain of a protein involving immune response to the hinge region of an Fc fragment of an immunoglobulin molecule. A simple fusion monomeric protein may be designated "protein name/Fc" for convenience in the present invention. For example, a simple fusion monomeric protein produced by linkage of an soluble extracellular domain of TNFR1 protein involving immune response to an Fc fragment of an immunoglobulin molecule is designated TNFR1/Fc. If desired, the origin of the Fc fragment may be also specified in the designation. For example, in the case that the Fc fragment is derived from IgG1, the monomeric protein is called TNFR1/IgG1Fc.

The term "simple fusion dimeric protein", as used herein, refers to a fusion protein having a dimeric structure, in which two simple fusion monomeric proteins are joined by formation of intermolecular disulfide bonds at the hinge region. Such a simple fusion dimeric protein may be designated "[protein name/Fc]$_2$" for convenience in the present invention. For example, when fused by formation of intermolecular disulfide

bonds at the hinge region of two simple fusion monomeric proteins produced by linkage of an soluble extracellular domain of TNFR1 protein and an Fc fragment of an immunoglobulin molecule, the resulting fusion protein having dimeric structure is designated $[TNFR1/Fc]_2$. In addition, the origin of the Fc fragment may be specified in the designation, if desired. For example, in the case that the Fc fragment is derived from IgG1, the dimeric protein is designated $[TNFR1/IgG1Fc]_2$.

The term "concatameric fusion monomeric protein", as used herein, refers to a fusion protein having a monomeric structure consisting of a single polypeptide, in which the N-terminus of a soluble extracellular domain of a protein involving immune response is linked to the C-terminus of an identical soluble extracellular domain of the protein involving immune response, wherein the C-terminus of the former soluble extracellular domain is linked to the hinge region of an Fc fragment of an immunoglobulin molecule. A concatameric fusion monomeric protein may be designated "protein name-protein name/Fc" for convenience in the present invention. For example, when an soluble extracellular domain of TNFR1 of a simple fusion monomeric protein, produced by linkage of the soluble extracellular domain of TNFR1 protein involving immune response and an Fc fragment of an immunoglobulin molecule, is linked to an identical soluble extracellular domain of TNFR1, the resulting concatameric fusion monomeric protein is designated TNFR1-TNFR1/Fc. If desired, the origin of the Fc fragment may be specified in the designation. For example, in the case that the Fc fragment is derived from IgG1, the monomeric protein is designated TNFR1-TNFR1/IgG1Fc.

The term "concatameric fusion dimeric protein", as used herein, refers to a fusion protein having a dimeric structure, in which two concatameric fusion monomeric proteins are fused by formation of intermolecular disulfide bonds at the hinge region. A concatameric fusion dimeric protein may be designated "[protein name-protein name/Fc]$_2$" for convenience in the present invention. For example, when two concatameric fusion monomeric proteins, each of which is produced by linkage of a TNFR1 soluble extracellular domain of a simple fusion monomeric protein to an identical soluble extracellular domain of TNFR1 protein involving immune response, are fused by formation of intermolecular disulfide bonds at the hinge region, the resulting fusion protein having dimeric structure is designated $[TNFR1\text{-}TNFR1/Fc]_2$, wherein the simple

fusion monomeric protein is formed by linkage of the TNFR1 soluble extracellular domain to an Fc fragment from an immunoglobulin molecule. If desired, the origin of the Fc fragment may be specified in the designation. For example, in the case that the Fc fragment is derived from IgG1, the fusion protein is designated [TNFR1-TNFR1/IgG1Fc]$_2$.

The term "vector", as used herein, means a DNA molecule serving as a vehicle capable of stably carrying exogeneous genes into host cells. For useful application, a vector should be able to replicate, have a system for introducing itself into a host cell, and possess selectable markers. The exogeneous genes, for example, include, a DNA construct encoding a concatameric fusion monomeric protein.

The term "recombinant expression plasmid", as used herein, refers to a circular DNA molecule carrying exogeneous genes operably linked thereto to be expressed in a host cell. When introduced into a host cell, the recombinant expression plasmid has the ability to replicate regardless of host chromosomal DNA, copy itself at a high copy number, and to produce heterogeneous DNA. As generally known in the art, in order to increase the expression level of a transfected gene in a host cell, the gene should be operably linked to transcription and translation regulatory sequences functional in a host cell selected as an expression system. Preferably, the expression regulation sequences and the exogeneous genes may be carried in a single expression vector containing bacteria-selectable markers and a replication origin. In case that eukaryotic cells are used as an expression system, the expression vector should further comprise expression markers useful in the eukaryotic host cells.

The term "operably linked", as used herein, means an arrangement of elements of a vector, in which each element is capable of performing its innate function. Therefore, a control sequence operably linked to a coding sequence can influence expression of the coding sequence. A control sequence acting to induce expression of a coding sequence does not have to be adjacent to the coding sequence. For example, when an intervening sequence is present between a promoter sequence and a coding sequence, the promoter sequence may still be "operably linked" to the coding sequence.

Host cells used in the present invention may be prokaryotic or eukaryotic. In addition, host cells having high introduction efficiency of foreign DNA and having high

expression levels of an introduced gene may be typically used. Examples of the host cells useful in the present invention include prokaryotic and eukaryotic cells such as *E. coli*, *Pseudomonas* sp., *Bacillus* sp., *Streptomyces* sp., fungi or yeast, insect cells such as *Spodoptera frugiperda* (Sf9), animal cells such as Chinese hamster ovary cells (CHO) or mouse cells, African green monkey cells such as COS 1, COS 7, human embryonic kidney cells, BSC 1, BSC 40 or BMT 10, and tissue-cultured human cells. When cloning a DNA construct encoding the fusion protein according to the present invention, host cells are preferably animal cells. When using COS cells, since SV40 large T antigen is expressed in COS cells, a plasmid carrying a SV 40 replication origin may be present as a multicopy episome and thus allows high expression of an exogeneous gene. A DNA sequence introduced into a host cell may be homogeneous or heterogeneous to the host cell, or a hybrid DNA sequence containing a homogenous or heterogeneous DNA sequence.

In order to express a DNA sequence encoding the concatameric fusion protein according to the present invention, a wide variety of combinations of host cells as an expression system and vectors may be used. Expression vectors useful for transforming eukaryotic host cells contain expression regulation sequences from, for example, SV40, bovine papillomavirus, adenovirus, adeno-associated viruses, cytomegalovirus and retroviruses. Expression vectors useful in bacterial host cells include bacterial plasmids from *E. coli*, which are exemplified by pBluescript, pGEX2T, pUC, pCR1, pBR322, pMB9 and derivatives thereof, plasmids having a broad range of host cells, such as RP4, phage DNAs, exemplified by a wide variety of λ phage derivatives including λ gt10, λ gt11 and NM989, and other DNA phages, exemplified by filamentous single-stranded DNA phages such as M13. Expression vectors useful in yeast cells include 2μ plasmid and derivatives thereof. Expression vectors useful in insect cells include pVL 941.

The term "transformation", as used herein, means introducing DNA into a suitable host cell so that the DNA is replicable, either as an extrachromosomal element, or by chromosomal integration.

The term "transfection", as used herein, refers to the taking up of an expression vector by a suitable host cell, whether any coding sequences are in fact expressed or not.

The term "signal sequence", as used herein, means an amino acid sequence mediating transport of an expressed protein to the outside of the cell membrane, and is also

called a "leader sequence". Cell surface proteins or secretory proteins, which are transported to the outside of the cell membrane, have an N-terminal sequence typically cut by signal peptidase in the cell membrane. Such a N-terminal sequence is called a signal sequence or signal peptide, or a leader sequence or leader peptide. Secretory (or transported) proteins or all proteins present outside of the cell membrane or in the extracellular environment have a specific signal sequence. There is no specific homology between such signal sequences and same proteins have different signal sequences according to their origin. Secondary structure or distribution of nonpolar and charged residues is more important for proper function of the signal sequences than primary structures thereof. Although not having specific homology, the signal sequences share several common features, as follows. The signal sequences contain an N domain at their N-termini, which is a hydrophilic region comprising one or more positively charged residues, and an H-domain follows the N domain, which is a somewhat long hydrophobic region. In the case of E. coli, the signal sequence comprises about 18-30 amino acids. The N domain contains many cationic amino acids such as Lys or Arg, and thus has a net positive charge. Many hydrophobic amino acids such as Ala or Leu are found in the H domain, and polar or charged amino acids such as Pro, Lys, Arg, Asn or Glu are rarely in the H domain. A large number of amino acids such as Ala and Leu residues form an α-helical structure to facilitate membrane penetration. A C domain is positioned between the H domain and an actually secreted portion of a protein. The C domain is less hydrophobic, and contains a sequence capable of being recognized by signal peptidase such as LebB or LspA. There have been no reports about an exact site cleaved by the signal peptidase, but the signal peptidase is typically known to mostly cleave behind the Ala-X-Ala sequence in the C domain. Preproteins containing the above-mentioned signal sequence arrive at the cell membrane through interaction with several proteins, and fold to their mature forms through cleavage of a specific region of a signal peptide. Such a signal sequence is very important in strategies to express a desired protein on the cell surface or in the extracellular environment. Foreign proteins and fusion proteins should be stably transported to the extracellular environment at high efficiency. Typically, cell surface proteins having excellent secretory ability are useful for cell surface expression of foreign proteins or fusion

proteins, which typically have secretory signal sequences capable of offering excellent secretion efficiency.

Preparation of the concatameric fusion dimeric protein according to the present invention

The concatameric fusion dimeric protein according to the present invention is generally prepared by (a) preparing a DNA construct encoding a simple fusion monomeric protein using a gene encoding an Fc fragment of an immunoglobulin molecule and a gene encoding a soluble extracellular domain of a protein involving immune response; (b) inserting by polymerase chain reaction (PCR) a recognition sequence of a restriction enzyme into the prepared simple fusion monomeric protein-encoding DNA construct and an identical gene to the gene encoding a soluble extracellular domain of a protein involving immune response, respectively; (c) cleaving the recognition sequence of a restriction enzyme in the simple fusion monomeric protein-coding DNA construct and the gene encoding a soluble extracellular domain of a protein involving immune response using the restriction enzyme recognizing the recognition sequence; (d) ligating the cleaved DNA fragments using ligase to produce a DNA construct encoding a concatameric fusion monomeric protein (see, Fig. 2); (e) operably linking the prepared DNA construct encoding a concatameric fusion monomeric protein to a vector to produce a recombinant expression plasmid; (f) transforming or transfecting a host cell with the recombinant expression plasmid; and (g) culturing the transformant or transfectant under conditions suitable for expression of the DNA construct encoding a concatameric fusion monomeric protein and then isolating and purifying a concatameric fusion dimeric protein of interest.

A DNA fragment encoding a soluble extracellular domain of a protein involving immune response is produced by PCR using a primer containing a recognition sequence of a specific restriction enzyme and a sequence encoding a leader sequence, and a primer containing an antisense sequence encoding the 3′ end of the soluble extracellular domain and a portion of the 5′ end of a specific region of Fc fragment of an immunoglobulin molecule.

A DNA fragment encoding a specific region of the Fc fragment of an immunoglobulin molecule is produced by PCR using a primer having a sequence encoding a portion of the 3′ end of the soluble extracellular domain of the protein involving immune response and a sequence encoding the 5′ end of the specific region of the Fc fragment of an immunoglobulin molecule, and another primer having an antisense sequence encoding a recognition sequence of a specific restriction enzyme and the 3′ end of a specific region of the Fc fragment of an immunoglobulin molecule.

The DNA fragment encoding a soluble extracellular domain of a protein involving the immune response and the DNA fragment encoding a specific region of Fc fragment of an immunoglobulin molecule, as described above, are mixed in a test tube. After denaturation, the DNA is re-annealed. Then, a complete double-stranded DNA fragment is produced by polymerization using DNA polymerase at the 3′ end of each DNA hybrid. Using the resulting double-stranded DNA fragment, another polymerase chain reaction (PCR) is carried out with the primer having a sequence encoding a soluble extracellular domain of a protein involving immune response and the primer encoding the 3′ end of a specific region of the Fc fragment of an immunoglobulin molecule, in order to amplify a immunoglobulin fusion gene comprising a sequence corresponding to the DNA fragment encoding a soluble extracellular domain of a protein involving immune response and a sequence corresponding to the DNA fragment encoding a specific region of the Fc fragment of an immunoglobulin molecule.

An recognition sequence of a restriction enzyme is introduced by PCR into the amplified immunoglobulin fusion gene and the DNA fragment having a sequence encoding a soluble extracellular domain of a protein involving the immune response. The recognition sequence is then cleaved with the restriction enzyme and the cleaved regions are ligated using ligase, thus producing a concatameric immunoglobulin fusion gene.

The immunoglobulin fusion gene may further include a signal sequence to stimulate extracellular secretion of a protein encoded thereby. For example, the CTLA-4 molecule contains a unique leader sequence having highly hydrophilic redundancy at its N-terminus, and which is abnormally long and highly water-soluble (Harper, K. et al., J. Immunol. 147:1037-1044; and Brunet, J.F. Nature 328:267-270, 1987). Generally, most

cell surface proteins or secretory proteins have a leader sequence comprising 20-24 highly hydrophobic amino acids at their N-termini. However, the CTLA-4 molecule used in the present invention comprises a total of 37 residues: 16 hydrophilic amino acids at its N-terminus, and 21 highly hydrophobic amino acids typical in its transmembrane regions. In the conventional method of preparing CTLA4Ig fusion proteins, the leader sequence of the CTLA-4 molecule was substituted with a leader sequence of oncostatin M (Linsley, P.S. et al., J. Exp. Med. 174:561-569, 1991) or IL-6 (Yamada, A, et al., Microbiol. Immunol. 40:513-518, 1996). The present inventors demonstrated that a CTLA-4 molecule containing a leader sequence having a "MRTWPCTLLFFIPVFCKA" sequence instead of the amino acid sequence consisting of 16 amino acids, "ACLGFQRHKAQKNLAA", is preferable, and the secretion of an expressed protein to the extracellular environment is easily achieved, as disclosed in International Pat. Publication No. WO98/31820.

A recombinant expression plasmid is prepared by inserting the immunoglobulin fusion gene into a vector, and then introduced to a host cell to produce a transformant or transfectant. A concatameric fusion dimeric protein of interest may be obtained by culturing the transformant or transfectant cell and isolating and purifying a concatameric fusion protein.

A host cell useful for preparation of the concatameric fusion dimeric protein according to the present invention is preferably selected from among bone marrow cell lines, CHO cells, monkey COS cells, human embryonic kidney 293 cells, and baculovirus-infected insect cells. A polypeptide of interest, produced in such an expression system, is secreted to culture medium as an inclusion body. Then, the concatameric fusion dimeric protein can be purified by affinity chromatography using a protein A or protein G column. In fact, effective mammalian expression systems and such purification systems are very useful in expressing proteins involving immune response in a dimeric form, and isolation of such proteins.

Preparation of the glycosylated concatameric fusion dimeric protein according to the present invention

Secretory proteins produced in eukaryotic cells as host cells are modified by glycosylation. Glycosylation is known to influence in vivo stability and functionality as well as physical properties of a protein. Therefore, a preferred aspect of the present invention includes facilitating production of a concatameric fusion dimeric protein of interest using recombinant DNA techniques and the above-mentioned animal cell lines as host cells, and linking additional sugar chains to a soluble extracellular domain of a protein involving immune response.

Two glycosylation patterns are known. One is O-linked glycosylation, in which an oligosaccharide is linked to a serine or threonine residue, and the other is N-linked glycosylation, in which an oligosaccharide is linked to asparagine residue. N-linked glycosylation occurs at a specific amino acid sequence, particularly, Asn-X-Ser/Thr, wherein X is any amino acid excluding proline. N-linked oligosaccharide has a structure distinct from O-linked oligosaccharide, and glycosylated residues found in the N-linked type also differ from the O-linked type. For example, N-acetylgalactosamine is invariably linked to serine or threonine in O-linked oligosaccharide, while N-acetylglucosamine is linked to asparagines in all of N-linked oligosaccharides. The O-linked oligosaccharides generally contain only 1-4 sugar residues. In contrast, the N-linked oligosaccharides comprise 5 or more sugar residues, essentially including N-acetylglucosamine and mannose.

In accordance with the present invention, to allow additional O-linked or N-linked glycosylation, one or more nucleotides in a DNA sequence encoding a soluble extracellular domain of a protein involving immune response are altered, and the resulting DNA is expressed in a suitable animal host cell to induce glycosylation using the host system. In accordance with an aspect of the present invention, the glycosylated concatameric fusion dimeric protein according to the present invention may be prepared by altering a DNA sequence encoding a soluble extracellular domain of a protein involving immune response to induce or increase N-linked glycosylation by adding the sequence Asn-X-Ser/Thr.

Alteration of a DNA sequence to introduce glycosylation may be performed according to the conventional method common in the art. In a preferred aspect of the present invention, to protect the concatameric fusion protein, especially the two soluble

extracellular domains, from attack of intercellular proteinases and thus increase its half-life in serum, a DNA construct encoding a multiglycosylated concatameric fusion monomeric protein may be prepared using PCR, which introduces multiglycosylation sites to the joint region between two soluble extracellular domains. In a specific aspect of the present invention, glycosylation motif peptide sequences may be introduced into the concatameric fusion protein, as follows. A DNA fragment is prepared by performing PCR using a primer encoding a leader sequence of a soluble extracellular domain and EcoRI restriction site, and an antisense primer in which a portion of a nucleotide sequence encoding a portion of the 3′ end of a first soluble extracellular domain and a portion of the 5′ end of a second soluble extracellular domain is substituted with glycosylation motif sequences. Another DNA fragment is prepared by performing PCR using a primer in which a portion of a nucleotide sequence encoding a portion of the 3′ end of a first soluble extracellular domain and a portion of the 5′ end of a second soluble extracellulular domain is substituted with glycosylation motif sequences, and an antisense primer encoding the 3′ end of Fc portion of IgG1 and XbaI restriction site. Then, secondary PCR is carried out in a test tube using the two DNA fragments.

In accordance with an embodiment of the present invention, the soluble extracellular domains useful in the present invention include soluble extracellular domains of TNFR1, TNFR2, CD2 and CTLA-4. Their application will be described in detail with reference to accompanying figures, sequence listing and examples.

Tumor necrosis factor-alpha (TNF-α), which is known as the hormone cachectin, and tumor necrosis factor-beta (TNF-β), which is also known as lymphotoxin, are multifunctional cytokines, inducing inflammation, cellular immune response, septicemia, cytotoxicity, cachexia, rheumatoid arthritis, inflammation-related diseases (Tartaglia, L.A. et al., Immunol. Today 13:151,1992), and antiviral reaction (Butler, P., Peptide Growth Factor II, 1990, Springer-Verlag, Berlin, pp.39-70). Such actions of TNF-α and TNF-β, including cytotoxic activity, originate from their binding to TNF receptors in a trimeric form (Eck, M.J. et al., J. Biol. Chem. 267:2119, 1992). As TNF receptors, 55 kDa-type I (TNFR1 or p55) and about 75 kDa-type II (TNFR2 or p75) are known (Smith, C.A. et al., Science 248:1019, 1990; Loetscher, H. et al., Cell 61:351, 1990; and Schall et al., Cell 61:361, 1990). The two receptors have similar affinity for TNF-α and TNF-β (Schall et

al., Cell 61:361, 1990). Immunoglobulin fusion proteins of such soluble receptors have effects of inhibiting the action of TNF-α and TNF-β by inhibiting binding of TNF-α and TNF-β to their receptors on the cell surface, which is known to be effective in reducing TNF-dependent inflammation.

Among cell surface antigens regulating immune response, the costimulatory molecule CD2 and CTLA-4, inducing secondary stimulation to give sufficient activation of T cells, when being in a soluble form, also can be used for therapy of diverse immunological diseases according to the same method as TNF receptors. Immune response is accomplished by binding of cell surface antigen molecules of antigen presenting cells (APC) to specific receptors of T lymphocytes, that is, T lymphocytes and leukocyte-function-antigen molecules of APC, and when a costimulatory signal as a secondary signal is not produced during antigen-presenting, T lymphocytes are removed by apoptosis or inhibition of clonal activation. CD2 is a leukocyte-function-antigen on T lymphocytes, binding to LFA-3 on APC, and participates in adhesion and costimulation of leukocytes, as well as stimulating T cell activation through costimulation with CD28. CTLA-4 is expressed after activation of T lymphocytes, and its expression level is increased in the resting phase. CTLA-4 has a binding affinity to the B7 molecule of APC over 20 times higher than that of CD28, and transduces signals inhibiting T lymphocyte activation after binding to B7.

In a specific aspect of the present invention, there are provided a concatameric fusion monomeric protein TNFR1-TNFR1/Fc, designated by SEQ ID NO: 6; a concatameric fusion monomeric protein TNFR2-TNFR2/Fc, designated by SEQ ID NO: 8; a concatameric fusion monomeric protein CD2-CD2/Fc, designated by SEQ ID NO: 18; and a concatameric fusion monomeric protein CTLA4-CTLA4/Fc, designated by SEQ ID NO: 20.

In another specific aspect of the present invention, there are provided a DNA construct (TNFR1-TNFR1-IgG) encoding a concatameric fusion monomeric protein TNFR1-TNFR1/Fc, designated by SEQ ID NO: 5; a DNA construct (TNFR2-TNFR2-IgG) encoding a concatameric fusion monomeric protein TNFR2-TNFR2/Fc, designated by SEQ ID NO: 7; a DNA construct (CD2-CD2-IgG) encoding a concatameric fusion monomeric protein CD2-CD2/Fc, designated by SEQ ID NO: 17; and a DNA construct

(CTLA4-CTLA4-IgG) encoding a concatameric fusion monomeric protein CTLA4-CTLA4/Fc, designated by SEQ ID NO: 19.

In a further specific aspect of the present invention, there are provided a recombinant expression plasmid pTR11Ig-Top10′ operably linked to a DNA construct encoding a concatameric fusion monomeric protein TNFR1-TNFR1/Fc, designated by SEQ ID NO: 5; a recombinant expression plasmid pTR22Ig-Top10′ operably linked to a DNA construct encoding a concatameric fusion monomeric protein TNFR2-TNFR2/Fc, designated by SEQ ID NO: 7; a recombinant expression plasmid pCD22Ig operably linked to a DNA construct encoding a concatameric fusion monomeric protein CD2-CD2/Fc, designated by SEQ ID NO: 17; and a recombinant expression plasmid pCT44Ig operably linked to a DNA construct encoding a concatameric fusion monomeric protein CTLA4-CTLA4/Fc, designated by SEQ ID NO: 19. The recombination expression plasmids are deposited in Korean Culture Center of Microorganisms (KCCM) and are assigned accession Nos. KCCM-10288, KCCM-10291, KCCM-10402 and KCCM-10400, respectively. The KCCM deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

In a further specific aspect of the present invention, there are provided a mammalian host cell (e.g., TR11Ig-CHO) transformed or transfected with a recombinant expression plasmid pTR11Ig-Top10′ operably linked to a DNA construct encoding a concatameric fusion monomeric protein TNFR1-TNFR1/Fc, designated by SEQ ID NO: 5; a mammalian host cell (e.g., TR22Ig-CHO) transformed or transfected with a recombinant expression plasmid pTR22Ig-Top10′ operably linked to a DNA construct encoding a concatameric fusion monomeric protein TNFR2-TNFR2/Fc, designated by SEQ ID NO: 7; a mammalian host cell transformed or transfected with a recombinant expression plasmid pCD22Ig operably linked to a DNA construct encoding a concatameric fusion monomeric protein CD2-CD2/Fc, designated by SEQ ID NO: 17; and a mammalian host cell transformed or transfected with a recombinant expression plasmid pCT44Ig operably linked to a DNA construct encoding a concatameric fusion monomeric protein CTLA4-CTLA4/Fc, designated by SEQ ID NO: 19. Chinese hamster ovary cell line TR11Ig-CHO transfected with the recombinant expression

plasmid pTR11Ig-Top10′ and Chinese hamster ovary cell line TR22Ig-CHO transfected with the recombinant expression plasmid pTR22Ig-Top10′ are deposited in KCCM and are assigned accession Nos. KCLRF-BP-00046 and KCLRF-BP-00049, respectively. The KCCM deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

In a still further specific aspect of the present invention, there are provided a concatameric fusion monomeric protein mgTNFR1-TNFR1/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 10; a concatameric fusion monomeric protein mgTNFR2-TNFR2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 12; a concatameric fusion monomeric protein mgCD2-CD2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 22; and a concatameric fusion monomeric protein mgCTLA4-CTLA4/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 24.

In a still further specific aspect of the present invention, there are provided a DNA construct encoding a concatameric fusion monomeric protein mgTNFR1-TNFR1/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 9; a DNA construct encoding a concatameric fusion monomeric protein mgTNFR2-TNFR2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 11; a DNA construct encoding a concatameric fusion monomeric protein mgCD2-CD2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 21; and a DNA construct encoding a concatameric fusion monomeric protein mgCTLA4-CTLA4/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 23. In order to produce a glycosylation motif peptide, a primer set (forward and reverse primers) is designed, which are complementary to a nucleotide sequence corresponding to the joint region between soluble extracellular domains of concatameric fusion proteins of TNFR/Fc, CD2/Fc and CTLA4/Fc, as well as containing codons encoding asparagine (N) (ATT and AAC) or codons encoding serine (S) and threonine (T) (TCC; and ACC, ACG and ACA, respectively), with which any codon in the concatameric fusion protein gene may be substituted. When designing the primer, selection of one among a plurality of amino acid sequences may be determined

depending on a condition allowing minimum substitution of the nucleotide sequence and melting temperature (T_m) of each primer.

In a still further specific aspect of the present invention, there are provided a recombinant expression plasmid pTR11Ig-MG operably linked to a DNA construct encoding a concatameric fusion monomeric protein mgTNFR1-TNFR1/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 9; a recombinant expression plasmid pTR22Ig-MG operably linked to a DNA construct encoding a concatameric fusion monomeric protein mgTNFR2-TNFR2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 11; a recombinant expression plasmid pCD22Ig-MG operably linked to a DNA construct encoding a concatameric fusion monomeric protein mgCD2-CD2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 21,; and a recombinant expression plasmid Pct44Ig-MG operably linked to a DNA construct encoding a concatameric fusion monomeric protein mgCTLA4-CTLA4/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 23. The recombination expression plasmids are deposited in Korean Culture Center of Microorganisms (KCCM) and are assigned accession Nos. KCCM-10404, KCCM-10407, KCCM-10401 and KCCM-10399, respectively. The KCCM deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure.

In a still further specific aspect of the present invention, there are provided a mammalian host cell transformed or transfected with a recombinant expression plasmid pTR11Ig-MG operably linked to a DNA construct encoding a concatameric fusion monomeric protein mgTNFR1-TNFR1/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 9; a mammalian host cell transformed or transfected with a recombinant expression plasmid pTR22Ig-MG operably linked to a DNA construct encoding a concatameric fusion monomeric protein mgTNFR2-TNFR2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 11; a mammalian host cell transformed or transfected with a recombinant expression plasmid pCD22Ig-MG operably linked to a DNA construct encoding a concatameric fusion monomeric protein mgCD2-CD2/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 21; and a mammalian host cell transformed or transfected with a recombinant expression plasmid

Pct44Ig-MG operably linked to a DNA construct encoding a concatameric fusion monomeric protein mgCTLA4-CTLA4/Fc containing glycosylation motif peptides, designated by SEQ ID NO: 23.

The concatameric fusion dimeric proteins of the present invention may be isolated from culture medium after culturing the transformants or transfectants according to the present invention. The concatameric fusion dimeric proteins may participate in immune response, as described in Table 1, above, and are thus useful as therapeutic agents, diagnostic agents and laboratory tools according to the kinds of the protein, and their use is well known to those of ordinary skill in the art. In particular, when being used as therapeutic agents, the concatameric fusion dimeric proteins may be applied at an therapeutically effective amount common in the art, and it will be understood that such an amount may vary depending on diverse factors including activity of the used compound, patient's age, body weight, health state, sex and diet, administration time, administration route, combination of drugs, and pathogenic state of a specific disease to be prevented or treated. In addition, when being used as therapeutic agents, it will be understood that the concatameric fusion dimeric proteins according to the present invention may be applied by the typical methods and routes for administration of proteins involving immune response, which are known to those of ordinary skill in the art.

The present invention will be explained in more detail with reference to the following examples in conjunction with the accompanying drawings. However, the following examples are provided only to illustrate the present invention, and the present invention is not limited to them. For convenience in describing the present invention, information on DNA constructs, recombinant expression plasmids and transformed cell lines, which are prepared according to the Examples, below, and the used primers and accession numbers is summarized in Tables 8 and 9, below.

TABLE 8

Information on DNA constructs and accession Nos.

DNA construct name	SEQ ID No.		Deposition of genes		Deposition of cell lines	
	DNA	Protein	Designation	Accession No.	Designation	Accession No.
TNFR1-IgG	1	2				
INFR2-IgG	3	4				
TNFR1-TNFR1-IgG	5	6	pTR11Ig-Top10′	KCCM 10288	TR11Ig-CHO	KCLRF-BP-00046
TNFR2-TNFR2-IgG	7	8	pTR22Ig-Top10′	KCCM 10291	TR22Ig-CHO	KCLRF-BP-00049
mgTNFR1-TNFR1-IgG	9	10	pTR11Ig-MG	KCCM 10404		
mgTNFR2-TNFR2-IgG	11	12	PTR22Ig-MG	KCCM 10407		
CD2-IgG	13	14				
CTLA4-IgG	15	16				
CD2-CD2-IgG	17	18	pCD22Ig	KCCM 10402		
CTLA4-CTLA4-IgG	19	20	pCT44Ig	KCCM 10400		
mgCD2-CD2-IgG	21	22	pCD22Ig-MG	KCCM 10401		
mgCTLA4-CTLA4-IgG	23	24	pCT44Ig-MG	KCCM 10399		

TABLE 9

Information for primers

Primer name	SEQ ID No.	Description
Oligo TNFR-EDF-EcoRI	25	Containing 5′ end of the extracellular domain of TNFR1 and an EcoRI site
Oligo TNFR-EDR-IgGh	26	Reverse primer containing 3′ end of the extracellular domain of TNFR1 and the hinge region of IgG
Oligo IgG1-T1F	27	Containing 5′ end of the hinge region of IgG and 3′ end of TNFR1
Oligo IgG1-R-XbaI	28	Reverse primer containing 3′ end of the hinge region of IgG and a XbaI site
Oligo TNFR2-EDF-EcoRI	29	Containing 5′ end of the extracellular domain of TNFR2 and an EcoRI site
Oligo TNFR2-EDR-IgGh	30	Reverse primer containing 3′ end of the extracellular domain of TNFR2 and the hinge region of IgG
Oligo IgG1-T2F	31	Containing 5′ end of the hinge region of IgG and 3′ end of TNFR2
Oligo TNFR1-CF-BamHI	32	Containing 5′ end of the extracellular domain of TNFR1 and a BamHI site; and used for preparation of a concatamer
Oligo TNFR1-NR-BamHI	33	Reverse primer containing 3′ end of the extracellular domain of TNFR1 and a BamHI site; and used for preparation of a concatamer
Oligo TNFR2-CF-BamHI	34	Containing 5′ end of the extracellular domain of TNFR2 and a BamHI site; and used for preparation of a concatamer
Oligo TNFR2-NR-BamHI	35	Reverse primer containing 3′ end of the extracellular domain of TNFR2 and a BamHI site; and used for preparation of a concatamer
Oligo mgTNFR1-TNFR1-IgG-F	36	Primer for mutagenesis, containing a sequence capable of inserting glycosylation sites into the joint region of TNFR1-TNFR1, and sequences corresponding to 3′ end and 5′ end of TNFR1; and used for preparation of a MG (multiglycosylation) form
Oligo mgTNFR1-TNFR1-IgG-R	37	Reverse primer for mutagenesis, containing a sequence capable of inserting glycosylation sites into the joint region of TNFR1-TNFR1, and sequences corresponding to 3′ end and 5′ end of TNFR1; and used for preparation of a MG form
Oligo mgTNFR2-TNFR2-IgG-F	38	Primer for mutagenesis, containing a sequence capable of inserting glycosylation sites into the joint region of TNFR2-TNFR2, and sequences corresponding to 3′ end and 5′ end of TNFR2; and used for preparation of a MG form
Oligo mgTNFR2-TNFR2-IgG-R	39	Reverse primer for mutation, containing a sequence capable of inserting glycosylation sites into the joint region of TNFR2-TNFR2, and sequences corresponding to 3′ end and 5′ end of TNFR2; and used for preparation of a MG form
Oligo CD2F-EcoRI	40	Containing 5′ end of the extracellular domain of CD2 and a EcoRI site
Oligo CD2R-RstI	41	Containing 3′ end of the extracellular domain of CD2 and a PstI site
Oligo IgG-F-PstI	42	Containing 5′ end of the hinge region of IgG and a PstI site
Oligo CTLA4F-EcoRI	43	Containing 5′ end of the extracellular domain of CTLA-4 and a EcoRI site
Oligo CTLA4R-PstI	44	Containing 3′ end of the extracellular domain of CTLA-4 and a PstI site
Oligo CD2-NT-F	45	Containing 5′ end of the extracellular domain of CD2; and used for preparation of a concatamer
Oligo CD2-CT-R	46	Reverse primer containing 3′ end of the extracellular domain of CD2; and used for preparation of a concatamer
Oligo CTLA4-NT-F	47	Containing 5′ end of the extracellular domain of CTLA-4; and used for preparation of a concatamer
Oligo CTLA4-CT-R	48	Reverse primer containing 3′ end of the extracellular domain of CTLA-4; and used for preparation of a concatamer
Oligo mgCD2-CD2-IgG-F	49	Used for preparation of a MG (multiglycosylation) form of CD2-CD2-IgG
Oligo mgCD2-CD2-IgG-R	50	Reverse primer used for preparation of a MG (multiglycosylation) form of CD2-CD2-IgG
Oligo mgCTLA4-CTLA4-IgG-F	51	Used for preparation of a MG (multiglycosylation) form of CTLA4-CTLA4-IgG
Oligo mgCTLA4-CTLA4-IgG-R	52	Reverse primer used for preparation of a MG (multiglycosylation) form of CTLA4-CTLA4-IgG

EXAMPLE 1

Human TNFR

A. Manufacture of a DNA construct encoding simple fusion monomeric protein of TNFR1/Fc (Fig. 1 and Fig. 5)

a. DNA fragment encoding soluble extracellular domain of TNFR1

A fusion gene encoding soluble extracellular domain of type I human TNF receptor (TNFR1, p55) and Fc fragment of human immunoglobulin G1 was constructed by the Polymerase Chain Reaction (PCR) method described in the prior art (Holten et al., Biotechniques 8:528, 1990).

A DNA fragment encoding soluble extracellular domain of TNFR1 was constructed by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 25) with EcoRI restriction site and the sequence encoding leader sequence (the sequence of amino acids 1-20 of SEQ ID NO: 2), and an antisense primer (the sequence of nucleotide of SEQ ID NO: 26) with the sequence encoding a part of 3' ends of the said soluble extracellular domain of TNFR1 (TNFR1-ED) and 5' ends of the hinge region of immunoglobulin G1 (IgG1). The template cDNA for this reaction was constructed by reverse transcription PCR (RT-PCR) of mRNA extracted from monocyte (T lymphocyte) of healthy adults.

After blood of healthy adults was extracted and diluted to 1:1 with RPMI-1640 (Gibco BRL, USA), the layer of T lymphocyte which formed at upper part was obtained by density gradient centrifugation using Ficoll-hypaque (Amersham, USA). In order to make the concentration of the cell to $5X10^5$ cells/ml, the cell was washed with RPMI-1640 for 3 times, and RPMI-1640 culture media containing 10% Fetal Bovine Serum (FBS, Gibco BRL, USA) was added, then cultured at 37℃ for two days in the 5% CO_2 incubator after adding leukoagglutinin to 3.5ug/ml (Pharmacia, USA).

The mRNAs were purified using Tri-Reagent (MRC, USA) mRNA purification kit. First, $2X10^7$ of human T lymphocyte was washed with Phosphate Buffered Saline (PBS, pH7.2) for 3 times, and then 1ml of Tri-Reagent was mixed for several times to dissolve RNA. After adding 0.2ml of chloroform to this tube and mixing thoroughly, this tube was incubated at room temperature (RT) for 15 min, then centrifuged at 15,000 rpm, 4℃ for 15 min. The upper part of the solution was transferred to a 1.5ml tube, and 0.5ml of isopropanol was added, and then centrifuged at 15,000 rpm, 4℃ for 15 min. After the supernatant was discarded, the pellet was resuspended with 1ml of 3° distilled water treated with 75% ethanol-25% DEPC (Sigma, USA), and then centrifuged at 15,000 rpm, 4℃ for 15 min. After the supernatant was removed completely and dried in the air to remove ethanol residue, RNA was resuspended with 50μl of 3° distilled water treated with DEPC.

The primary cDNA was synthesized by mixing 2μg of purified mRNA and 1μl of oligo dT (dT30, Promega, USA) primer to 10μM in 1.5ml tube, heating at 70℃ for 2 min, and cooling in ice for 2 min. After that, this mixture was added with 200U of M-MLV reverse transcriptase (Promega, USA), 10μl of 5 x reaction buffer (250mM Tris-HCl, pH 8.3, 375mM KCl, 15mM $MgCl_2$, and 50mM DTT), 1μl of dNTP (10mM each, Takara, Japan), and DEPC-treated 3° distilled water to 50μl, then reacted at 42℃ for 1 hour.

b. DNA fragment encoding Fc fragment of immunoglobulin

A DNA fragment encoding Fc fragment of immunoglobulin G1 was constructed by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 27) with the sequence encoding a part of 3' ends of the said soluble extracellular domain of TNFR and 5' end of the hinge region of immunoglobulin G1 (IgG1), and an antisense primer (the sequence of nucleotide of SEQ ID NO: 28) with XbaI restriction site and the sequence encoding 3' ends of IgG1 Fc. The template cDNA for this reaction was constructed by RT-PCR of mRNA extracted from peripheral blood cell (B lymphocyte) of convalescent patients with pyrexia of unknown origin.

c. DNA construct encoding simple fusion monomeric protein of TNFR1/Fc

After DNA fragment encoding soluble extracellular domain of TNFR1 and DNA fragment encoding Fc fragment of immunoglobulin produced as described above were mixed in the same tube, complementary binding between the common sequence (the sequence including 3' end of soluble extracellular domain of TNFR1 and 5' end of IgG1 hinge region) was induced. Using this mixture as a template, DNA construct including DNA fragment encoding soluble extracellular domain of TNFR1 and DNA fragment encoding IgG1 Fc fragment was amplified by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 25) with the sequence encoding 5' end of TNFR1 and another primer (the sequence of nucleotide of SEQ ID NO: 28) with the sequence encoding 3' end of IgG1 Fc. The constructed gene included a leader sequence to faciliate secretion of protein after expression.

d. Cloning of the DNA construct encoding simple fusion monomeric protein of TNFR1/Fc

DNA construct encoding simple fusion monomeric protein of TNFR1/Fc as described above was restricted with EcoRI and XbaI, and cloned by inserting into a commercially available cloning vector, pBluescript KS II (+) (Stratagene, USA), at EcoRI/XbaI site. The sequence of a total coding region was identified by DNA sequencing (SEQ ID NO: 1). This produced fusion protein was designated TNFR1/Fc as simple fusion monomeric protein, and the elliptical shape shown in Figure 1 represents the structure of a primary expression product of the fusion gene. The deduced amino acid sequence of simple fusion monomeric of TNFR1/Fc corresponded to SEQ ID NO: 2.

B. Manufacture of a DNA construct encoding simple fusion monomeric protein of TNFR2/Fc (Fig. 1 and Fig. 5)

a. DNA fragment encoding soluble extracellular domain of TNFR2

A fusion gene encoding soluble extracellular domain of type II human TNF receptor (TNFR2, p75) and Fc fragment of human immunoglobulin G1 was constructed by the same method as that of TNFR1/Fc.

A DNA fragment encoding soluble extracellular domain of TNFR2 was constructed by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 29) with EcoRI restriction site and the sequence encoding leader sequence (the sequence of amino acids 1-22 of SEQ ID NO: 4), and an antisense primer (the sequence of nucleotide of SEQ ID NO: 30) with the sequence encoding a part of 3' ends of said soluble extracellular domain of TNFR2 (TNFR2-ED) and 5' ends of the hinge region of immunoglobulin G1 (IgG1). The template cDNA for this reaction was constructed by RT-PCR of mRNA extracted from monocyte (T lymphocyte) of healthy adults.

b. DNA construct encoding simple fusion monomeric protein of TNFR2/Fc

After DNA fragment encoding soluble extracellular domain of TNFR2 and DNA fragment encoding Fc fragment of immunoglobulin G1 produced as described above were mixed in the same tube, complementary binding between the common sequence (the sequence including 3' end of soluble extracellular domain of TNFR2 and 5' end of IgG1 hinge region) was induced. Using this mixture as a template, DNA construct including DNA fragment encoding soluble extracellular domain of TNFR2 and encoding and DNA fragment encoding IgG1 Fc fragment was amplified by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 29) with the sequence encoding 5' end of TNFR2 and another primer (the sequence of nucleotide of SEQ ID NO: 28) with the sequence encoding 3' end of IgG1 Fc. The constructed gene includes a sequence to faciliate secretion of protein after expression.

c. Cloning of the DNA construct encoding simple fusion monomeric protein of TNFR2/Fc

DNA construct encoding simple fusion monomeric protein of TNFR2/Fc as described above was restricted with EcoRI and XbaI, and cloned by inserting into a commercially available cloning vector, pBluescript KS II (+) (Stratagene, USA), at EcoRI/XbaI site. The sequence of a total coding region was identified by DNA sequencing (SEQ ID NO: 3). This produced fusion protein was designated TNFR2/Fc as simple fusion monomeric protein, and the elliptical shape shown in Figure 1 represents the structure of a primary expression product of the fusion gene. The deduced amino acid sequence of simple fusion monomeric of TNFR2/Fc corresponded to SEQ ID NO: 4.

C. Manufacture of a DNA construct encoding concatameric fusion monomeric protein of TNFR1-TNFR1/Fc (Fig. 2 and Fig. 5)

In order to manufacture a fusion gene comprising the concatameric shape in soluble extracellular domain of TNFR1, i.e. the DNA construct encoding concatameric fusion monomeric protein of TNFR1-TNFR1/Fc, BamHI restriction site was inserted respectively into the sequence of soluble extracellular domain of TNFR1 and DNA construct as produced as above encoding simple fusion monomeric protein of TNFR1/Fc by PCR, and then regions of each fragments restricted by BamHI were linked by ligase. The DNA construct, encoding simple fusion monomeric protein of TNFR1/Fc produced as above, was used as the template of this reaction.

The fragment of the soluble extracellular domain of TNFR1 with BamHI restriction site at 3' end was amplified by PCR using a primer corresponding to the nucleotide of SEQ ID NO: 25 and another primer corresponding to the nucleotide sequence of SEQ ID NO: 33, and the other fragment of simple fusion monomeric protein of TNFR1/Fc with BamHI restriction site at 5' end was amplified by PCR using a primer

corresponding to the nucleotide of SEQ ID NO: 28 and another primer corresponding to the nucleotide sequence of SEQ ID NO: 32, respectively. PCR was performed by adding 1μl of primary cDNA, 2U of Pfu DNA polymerase (Stratagene, USA), 10μl of 10X reaction buffer [200mM Tris-HCl, pH 8.75, 100mM $(NH_4)_2SO_4$, 100mM KCl, 20mM $MgCl_2$], 1% Triton™ X-100, 1mg/ml BSA, 3μl primer 1 (10μM), 3μl primer 2 (10μM), 2μl dNTP (10mM each), and 3° distilled water to 100μl. The reaction condition was as follows; 94℃, 5 min; 95℃, 1 min; 58℃, 1 min 30 sec; 72℃, 1 min for 31 cycles; and 72℃, 15 min to make PCR product with complete blunt end.

After electrophorized on 0.8% agarose gel, the PCR product was purified by Qiaex II gel extraction kit (Qiagen, USA). The purified PCR product was restricted by BamHI and extracted by phenol-chloroform extraction methods. Subsequently, two kinds of DNA fragments restricted by BamHI were linked by ligase.

D. Manufacture of a DNA construct encoding concatameric fusion monomeric protein of TNFR2-TNFR2/Fc (Fig. 2 and Fig. 5)

After a BamHI restriction site was inserted respectively into the sequence of the soluble extracellular domain of TNFR21 and the DNA construct produced as described above encoding simple fusion monomeric protein of TNFR2/Fc by PCR, a DNA construct encoding concatameric fusion monomeric protein of TNFR2-TNFR2/Fc was manufactured by linking the regions of each fragments restricted by BamHI by ligase.

A fragment of soluble extracellular domain of TNFR2 with BamHI restriction site at 3' end was amplified using a primer corresponding the sequence of SEQ ID NO: 34 and SEQ ID NO: 35. PCR was performed as that of TNFR1, except that a DNA construct encoding simple fusion monomeric protein of SEQ ID NO: 3 produced as above was used as a template. The PCR product was purified by the method as that of TNFR1.

E. DNA construct encoding concatameric fusion monomeric protein of TNFR1-TNFR1/Fc with glycosylation motif.

A DNA fragment was manufactured by PCR using an antisense primer (the sequence of nucleotide of SEQ ID NO: 37) with the sequence encoding the part (the sequence of nucleotide 565-591 of SEQ ID NO: 5) of 3' end of the first soluble extracellular domain of TNFR1, except the sequence of hydrophobic peptide region (the sequence of amino acid 197-216 of SEQ ID NO: 6) at the junction of soluble extracellular domain of TNFR1 and the part (the sequence of nucleotide 649-681 of SEQ ID NO: 5) of 5' end of the second soluble extracellular domain of TNFR1, and another primer (the sequence of nucleotide of SEQ ID NO: 25) with the sequence encoding EcoRI restriction site and leader sequence.

In addition, the total four amino acid sequences encoding glycosylation site (the sequence of amino acids 189-191, 192-194, 198-200, and 204-206 of SEQ ID NO: 10) were inserted by manufacturing the primer as above (the sequence of nucleotide of SEQ ID NO: 36 and 37) corresponding the substitution of the nucleotide 565-567 (CTG, Leu), 574-576 (ACG, Thr), 652-654 (CTA, Leu), and 670-672 (AGA, Arg) of SEQ ID NO: 5 with the nucleotide of AAC (Asn, N); the nucleotide of 571-573 (TGC, Cys) and 580-582 (TTG, Leu) of SEQ ID NO: 5 with the nucleotide of ACC (Thr, T); the nucleotide of 658-660 (GAC, Asp) with the nucleotide of TCC (Ser, S).

In this reaction, the gene (the nucleotide of SEQ ID NO: 5) encoding concatameric shape of TNFR1-TNFR1/Fc was used as a template. During the primary PCR, only the half of the antisense primer was induced to bind the gene encoding concatameric shape of TNFR1-TNFR1/Fc used as a template, and, as chain reaction was proceeding, the unbound part to the template was induced to form a complete double-stranded DNA by polymerase, and then this was capable of producing the DNA fragment with state of linkage of the sequence of 5' end encoding the part of the second soluble

extracellular domain and the sequence of 3' end encoding TNFR1 extracellular domain including leader sequence. Therefore, a part of the sequence of 5' end encoding the second soluble extracellular domain has the function that was capable of binding to the second DNA fragment as follows.

The second DNA fragment was manufactured by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 36) with the sequence encoding the part (the sequence of nucleotide 565-591 of SEQ ID NO: 5) of 3' end of the first soluble extracellular domain of TNFR1 and the part (the sequence of nucleotide 649-681 of SEQ ID NO: 5) of 5' end of the second soluble extracellular domain of TNFR1, and an antisense primer (the sequence of nucleotide of SEQ ID NO: 28) with the sequence encoding a XbaI restriction site and 3' end of IgG1 Fc. This reaction was also performed as described above, that is, only the half of antisense primer was induced to bind the template, and consequently, DNA fragment like that described above had the sequence encoding 5' end of TNFR1 extracellular including the part of 3' end of the first soluble extracellular domain.

Subsequently, resulting from two kinds of DNA fragments as PCR described as above were mixed in the same tube, induced to bind between common sequences, and fused by PCR using primers (the sequence of nucleotide of SEQ ID NO: 25 and 28) encoding 5' and 3' end of each concatameric genes, and the product was designated mgTNFR1-TNFR1-IgG.

F. DNA construct encoding concatameric fusion monomeric protein of TNFR2-TNFR2/Fc with glycosylation motif.

A DNA fragment was manufactured by PCR using an antisense primer (the sequence of nucleotide of SEQ ID NO: 39) with the sequence encoding the part (the sequence of nucleotide 586-606 of SEQ ID NO: 7) of 3' end of first soluble extracellular domain of TNFR2, except the sequence of hydrophobic peptide region (the sequence of

amino acid 203-263 of SEQ ID NO: 8) at the junction of soluble extracellular domain of TNFR2 and the part (the sequence of nucleotide 790-807 of SEQ ID NO: 7) of 5' end of second soluble extracellular domain of TNFR2, and another primer (the sequence of nucleotide of SEQ ID NO: 29) with the sequence encoding EcoRI restriction site and leader sequence.

In addition ,the total two amino acid sequences encoding glycosylation site (the sequence of amino acids 199-201 and 206-208 of SEQ ID NO: 12) were inserted by manufacturing the primer as described above (the sequence of nucleotide of SEQ ID NO: 38 and 39) corresponding to the substitution of the nucleotide 595-597 (GTC, Val) and 799-801 (GGG, Gly) SEQ ID NO: 7 with the nucleotide of AAC (Asn, N).

In this reaction, the gene (the nucleotide of SEQ ID NO: 7) encoding concatameric shape of TNFR2-TNFR2/Fc was used as a template. During the primary PCR, only the half of antisense primer was induced to bind the gene encoding concatameric shape of TNFR2-TNFR2/Fc used as a template, and, as the chain reaction was proceeding, the unbound part to the template was induced to form a complete double-stranded DNA by polymerase, and thus this was capable of producing the DNA fragment with a state of linkage of the sequence of 5' end encoding the part of the second soluble extracellular domain and the sequence of 3' end encoding TNFR2 extracellular domain including the leader sequence. Therefore, a part of the sequence of 5' end encoding the second soluble extracellular domain has the function that was capable of binding to the second DNA fragment as follows.

The second DNA fragment was manufactured by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 38) with the sequence encoding the part (the sequence of nucleotide 586-606 of SEQ ID NO: 7) of 3' end of the first soluble extracellular domain of TNFR2 and the part (the sequence of nucleotide 790-807 of SEQ ID NO: 7) of 5' end of the second soluble extracellular domain of TNFR2, and an antisense primer (the sequence of nucleotide of SEQ ID NO: 28) with the sequence encoding a XbaI restriction

site and 3' end of IgG1 Fc. This reaction was also performed, that is, only the half of antisense primer was induced to bind the template, and consequently, DNA fragment like that described above had the sequence encoding 5' end of TNFR2 extracellular including the part of 3' end of first soluble extracellular domain.

Subsequently, resulting from two kinds of DNA fragments as PCR produced as above were mixed in the same tube, induced to bind between common sequences, and fused by PCR using primers (the sequence of nucleotide of SEQ ID NO: 29 and 28) encoding 5' and 3' end of each concatameric genes, and the product was designated mgTNFR2-TNFR2-IgG.

G. Cloning of DNA constructs encoding concatameric fusion monomeric protein of TNFR-TNFR/Fc and their glycosylated forms

DNA constructs encoding concatameric fusion monomeric protein of TNFR-TNFR/Fc and their glycosylated forms as above were cloned by inserting into pBluescript KS II (+) (Stratagene, USA) at EcoRI/XbaI site. These produced fusion proteins were designated TNFR1-TNFR1/Fc and TNFR2-TNFR2/Fc as concatameric fusion monomeric protein, and designated mgTNFR1-TNFR1/Fc and mgTNFR2-TNFR2/Fc as their glycosylated forms. The deduced amino acid sequences corresponded to SEQ ID NO: 6, 8, 10, and 12.

After 10μg of pBluescript KS II (+) (Stratagene, USA) used as a vector was mixed with 15U of EcoRI, 15U of XbaI, 5μl of 10X reaction buffer (100mM Tris-HCl, pH 7.5, 100mM $MgCl_2$, 10mM DTT, 500nM NaCl), 5μl of 0.1% BSA (Takara, Japan), and 3° distilled water to 50μl, DNA was restricted by incubation at 37℃ for 2 hrs. After electrophorized on 0.8% agarose gel, the PCR product was purified by Qiaex II gel extraction kit (Qiagen, USA).

After 100ng of pBluescript KS II (+) (Stratagene, USA) restricted by EcoRI and XbaI was mixed with 20ng of PCR product restricted by the restriction enzyme, 0.5U of T4 DNA ligase (Amersham, USA), 1μl of 10X reaction buffer (300mM Tris-HCl, pH 7.8, 100mM $MgCl_2$, 100mM DTT, 10mM ATP) and 3° distilled water were added to 10μl, and the mixture was incubated in the water bath at 16℃ for 16 hrs. E. coli Top10 (Novex, USA) was made to competent cell by the method of rubidium chloride (RbCl, Sigma, USA) and transformed, then spread on the solid LB media including 50μg/ml of ampicillin (Sigma, USA) and incubated at 37℃ for 16 hrs. Formed colonies were inoculated in 4ml of liquid LB media including 50μg/ml of ampicillin and incubated at 37℃ for 16 hrs. Plasmid was purified by the method of alkaline lysis according to Sambrook et al. (Molecular cloning, Cold Spring Harbor Laboratory press, p1.25-1.31, p1.63-1.69, p7.26-7.29, 1989) from 1.5ml of that, and the existence of cloning was confirmed by the restriction of EcoRI and XbaI.

The sequence of a total coding region was identified by the DNA sequencing method of dideoxy chain termination method (Sanger et al., Proc. Natl. Acad. Sci., 74:5483, 1977) as follows. The DNA sequencing reaction was performed according to the manual using a plasmid purified by alkaline lysis method as described above and Sequenase™ ver 2.0 (Amersham, USA). After the reaction mixture as above was loaded on 6% polyacrylamide gel and electrophorized for 2 hrs at constant voltage of 1,800~2,000 V and 50℃, DNA sequence was identified by exposing to X-ray film (Kodak, USA) after the gel was dried out.

EXAMPLE 2 AND 3

CD2 and CTLA4

DNA fragments encoding soluble extracellular domain of CD2 and CTLA4 were constructed by PCR using a primer [CD2(the sequence of nucleotide of SEQ ID NO:

40), and CTLA4(the sequence of nucleotide of SEQ ID NO: 43)] with EcoRI restriction site and the coding sequence [CD2 (the sequence of nucleotide of SEQ ID NO: 13), and CTLA4 (the sequence of nucleotide of SEQ ID NO: 15)] encoding the leader sequence [CD2(the sequence of amino acid 1-24 of SEQ ID NO: 14), and CTLA4(the sequence of amino acid 1-21 of SEQ ID NO: 16)], and an antisense primer [CD2(the sequence of nucleotide of SEQ ID NO: 41), and CTLA4(the sequence of nucleotide of SEQ ID NO: 44)] with PstI restriction site and the sequence [CD2(the sequence of nucleotide of SEQ ID NO: 13), and CTLA4(the sequence of nucleotide of SEQ ID NO: 15)] encoding 3' end of the soluble extracellular domain of the proteins as described above. The template cDNA for this reaction was constructed by reverse transcription PCR (RT-PCR) of mRNA extracted from the monocyte (T lymphocyte) of healthy adults.

Also, a DNA fragment encoding Fc fragment of immunoglobulin G1 was constructed by PCR using a primer (the sequence of nucleotide of SEQ ID NO: 42) with PstI restriction site and the sequence encoding 5' ends of constant region of IgG1, and an antisense primer (the sequence of nucleotide of SEQ ID NO: 28) with XbaI restriction site and the sequence encoding 3' ends of IgG1 Fc. The template cDNA for this reaction was constructed by RT-PCR of mRNA extracted from peripheral blood cell (B lymphocyte) of convalescent patients with unknown fever.

Subsequently, both DNA fragment encoding soluble extracellular domain of CD2 and CTLA4 and DNA fragment encoding Fc fragment of immunoglobulin G1 produced as described above were restricted by PstI, and then the simple dimeric shape of CD2/Fc and CTLA4/Fc genes were constructed by linkages using T4 DNA ligase. The constructed genes included a leader sequence to faciliate secretion of protein after expression.

DNA constructs as described above were restricted by restriction enzyme of EcoRI and XbaI, and cloned by inserting into a commercially available cloning vector, pBluescript KS II (+) (Stratagene, USA) at EcoRI/XbaI site. The sequence of a total coding

region was identified by DNA sequencing (SEQ ID NO: 13 and 15). These produced fusion proteins were designated CD2/Fc and CTLA4/Fc, and the deduced amino acid sequences of these corresponded to SEQ ID NO: 14 and 16.

PCR was performed by adding 1μl of primary cDNA, 2U of Pfu DNA polymerase (Stratagene, USA), 10μl of 10X reaction buffer [200mM Tris-HCl, pH 8.75, 100mM $(NH_4)_2SO_4$, 100mM KCl, 20mM $MgCl_2$], 1% Triton™ X-100, 1mg/ml BSA, 3μl primer 1 (10μM), 3μl primer 2 (10μM), 2μl dNTP (10mM each), and 3° distilled water to 100μl. The reaction condition was as follows; 94℃, 5 min; 95℃, 1 min; 58℃, 1 min 30 sec; 72℃, 1 min for 31 cycles; and 72℃, 15 min to make PCR product with complete blunt end.

The fusion genes with concatameric shape of CD2-CD2/Fc and CTLA4-CTLA4/Fc were constructed as follows.

In order to manufacture fusion gene comprising the concatameric shape in soluble extracellular domain of CD2 and CTLA4, the sequences of soluble extracellular domain of CD2 and CTLA4 were inserted by blunt-end ligation using ligase at the junction between extracellular domain and immunoglobulin of fusion genes in the shape of simple dimer with blunt end, using PstI restriction enzyme and T4 DNA polymerase. Specifically, DNA constructs were constructed by PCR using a primer [CD2(the sequence of nucleotide of SEQ ID NO: 13) and CTLA4(the sequence of nucleotide of SEQ ID NO: 48)] with the coding sequence [CD2(the sequence of nucleotide of SEQ ID NO: 13) and CTLA4(the sequence of nucleotide of SEQ ID NO: 15)] encoding the end of leader sequence [CD2(the sequence of amino acid 25 of SEQ ID NO: 14) and CTLA4(the sequence of amino acid 22 of SEQ ID NO: 16)] of soluble extracellular domain, and an antisense primer [CD2(SEQ ID NO: 46) and CTLA4(SEQ ID NO: 48)] with the sequence [CD2(the sequence of nucleotide of SEQ ID NO: 13) and CTLA4(the sequence of nucleotide of SEQ ID NO: 15)] encoding 3' end of soluble extracellular domain as above. The simple fusion monomeric genes [CD2/Fc (the sequence of nucleotide of SEQ ID NO: 13) and CTLA4/Fc (the sequence of

nucleotide of SEQ ID NO: 15)] described as above were used as the template of this reaction.

Also, CD2/Fc and CTLA4/Fc, which were inserted in pBluescript KS II (+) in the shape of simple monomeric form, were made to have 3' overhang end using the restriction enzyme of PstI. The cut end of 3' overhang was partially deleted to form a blunt end by treating T4 DNA polymerase. In order to manufacture fusion genes in the shape of concatamer in soluble extracellular domain, the soluble extracellular domains of CD2 and CTLA4 produced by PCR as described above were cloned by inserting into cut ends of simple monomeric gene made as blunt end. These produced fusion proteins were designated CD2-CD2/Fc and CTLA4-CTLA4/Fc as concatameric fusion monomeric protein, and their deduced amino acid sequences corresponded SEQ ID NO: 18 and 20, respectively.

The concatameric fusion genes in the shape of multiglycosylated form were constructed as follows.

The glycosylation mofit was inserted by secondary PCR with mixing in the same tube of a DNA fragment produced by PCR using a primer including EcoRI restriction site and the soluble extracellular domain with leader sequence, and an antisense primer with the sequence encoding the part of 3' end of the first soluble extracellular domain of concatameric shape of fusion gene and the part of 5' end of the second soluble extracellular domain with the nucleotide of substituted glycosylation motif; and other DNA fragment produced by PCR using a primer with the sequence encoding the part of 3' end of the first soluble extracellular domain of concatameric shape of fusion gene and the part of 5' end of the second soluble extracellular domain with the nucleotide of substituted glycosylation motif, and an antisense primer with the sequence encoding 3' end of Fc fragment of immunoglobulin G1 and XbaI restriction site.

In the case of concatameric fusion gene of CD2/Fc and CTLA4/Fc, the glycosylation motif was inserted by PCR using modified primers as the same methods as

that of TNFR/Fc described as above, but it was different from the case of TNFR/Fc that the amino acid sequence of binding to soluble extracellular domain of CD2 and CTLA4 was retained as the same.

In the process of multiglycosylatin of the concatameric fusion protein of CD2/Fc and CTLA4/Fc, the case of CD2/Fc was completed by inserting the total two glycosylation motif peptide region (the sequence of amino acid of 200-202 and 206-208 of SEQ ID NO: 22) using a manufactured primer including the substitution of the nucleotide of 598-600 (CCT, Pro) and 616-618 (GAG, Glu) of SEQ ID NO: 17 with AAT (Asn, N), and the case of CTLA4/Fc was completed by inserting the total three glycosylation motif peptide region (the sequence of amino acid of 136-138, 142-144, and 147-149 of SEQ ID NO: 24) using a manufactured primer(SEQ ID NO: 51 and 52) including the substitution of the nucleotide of 403-405 (GTA, Val) and 424-426 (CCA, Pro) of SEQ ID NO: 19 with AAT (Asn, N); the nucleotide of 409-411 (GAT, Asp) and 445-447 (GTG, Val) with ACA (Thr, T) and ACG (Thr, T), respectively. These produced fusion proteins were designated mgCD2-CD2/Fc and mgCTLA4-CTLA4/Fc as concatameric fusion monomeric protein, and their deduced amino acid sequences corresponded to SEQ ID NO: 22 and 24, respectively.

EXAMPLE 4

Expression and purification of simple/concatameric fusion dimeric protein of TNFR/Fc

In order to express the fusion proteins in CHO-K1 cell (ATCC CCL-61, Ovary, Chinese hamster, Cricetulus griseus), after pBluescript KS II (+) plasmid DNA including TNFR/Fc fusion gene was purified from transformed E. coli, an animal cell expression vectors were constructed as TNFR/Fc fragment produced by restriction using EcoRI and XbaI was inserted at EcoRI/XbaI site of an animal cell expression vector, pCR^{TM}3

(Invitrogen, USA) plasmid. And these were designated plasmid pTR11-Top10' and plasmid pTR22-Top10', and deposited as accession numbers of KCCM 10288 and KCCM 10291, respectively, at Korean Culture Center of Microorganisms (KCCM) on Jul. 10. 2001.

Transfection was performed by mixing either the plasmid pTR11-Top10' or plasmid pTR22-Top10' DNA including TNFR/Fc fusion genes as described above with the reagent of Lipofectamin™ (Gibco BRL, USA). CHO-K1 cells with the concentration of 1~3 X 10^5 cells/well were inoculated in 6-well tissue culture plate (Nunc, USA), and incubated to 50~80% in 10% FBS - DMEM media, then the DNA-liposome complex, which was reacted for 15~45 min with 1~2μg of either the plasmid pTR11-Top10' or plasmid pTR22-Top10' DNA including TNFR/Fc fusion genes as described above and 2~25μl of Lipofectamin™ (Gibco BRL, USA), were added to the cell culture plate in the serum-free DMEM media. After incubation for 5 hrs, DMEM media with 20% serum was added and cells were incubated further for 18~24 hrs. After primary transfection, cells were incubated for 3 weeks in 10% FBS - DMEM media with 1.5mg/ml of Geneticin (G418, Gibco BRL, USA), and formed colonies was selected for amplified incubation. The expression of fusion proteins was analyzed by ELISA using a peroxidase labeled goat anti-human IgG (KPL, USA).

ELISA was performed as follows. First, 1mg/ml of a peroxidase labeled goat anti-human IgG (KPL, USA) was diluted to 1:2,000 with 0.1M sodium bicarbonate, 100 μl of that was aliquoted into 96-well flexible plate (Falcon, USA) and sealed with plastic wrap, then incubated at 4℃ over 16 hrs to be coated on the surface of the plate. After this, it was washed for 3 times with washing buffer (0.1% Tween-20 in 1X PBS) and dilution buffer (48.5ml 1XPBS, 1.5ml FBS, 50ul Tween-20), and then was aliquoted to 180l. After 20μl of culture supernatant was dropped in the first well, then serially diluted using a micropipette, and 0.01㎍/㎕ of human immunoglobulin G (Sigma, USA) as the positive control and the culture media of untransfected CHO K-1 cell as the negative was equally diluted. After dilution, 96-well ELISA plate (Falcon, USA) was wrapped with aluminum

foil and incubated at 37℃ for 1 hr 30 min, washed for 3 times with washing buffer. Peroxidase conjugated goat anti-human IgG (KPL, USA) was diluted to 1:5,000 with dilution buffer, aliquoted to 100μl, wrapped with aluminum foil, and reacted at 37℃ for 1 hr. After reaction, this plate was washed for 3 times, colorized using TMB microwell peroxidase substrate system (KPL, USA) and existence of expression was confirmed by measurement of absorbance at 655nm wavelength using microplate reader (Bio-Rad, Model 550, Japan).

Transfectants manufactured as above were designated TR11Ig-CHO and TR22Ig-CHO and deposited as accession numbers of KCLRF-BP-00046 and KCLRF-BP-00049, respectively, at Korean Cell Line Research Foundation (KCLRF) on Jul. 7. 2001. And adaptation for transfectants as described above to one of the serum free media, CHO-S-SFM II (Gibco BRL, USA), was proceeded to purify the proteins produced by those transfectants as follows. After about $3X10^5$ of cells were inoculated into the 6-well plate, cells were cultured at 5% CO2, 37℃ for over 16 hrs to adhere, and it was checked under a microscope that cells were adhered at about 30~50% area of the plate, then cells were cultured in a media consisting of 10% FBS DMEM and CHO-S-SFM II in the ratio of 8:2. After culturing 3 times serial passage at this ratio, it was cultured 3 times at the ratio of 6:4; 3 times at 4:6; 3 times at 3:7; 3 times at 2:8; 3 times at 1:9; and finally cultured in 100% CHO-S-SFM II media. And the level of expression was measured by ELISA.

After these transfectant cells were cultured on a large scale in CHO-S-SFM II, the supernatants including each fusion proteins were centrifuged at 200X g for 12min to remove cell debris, and proteins were purified by the method using HiTrap protein A column (Amersham, USA) as follows. After 20mM of sodium phosphate (pH 7.0, Sigma, USA) was passed at the velocity of 1ml/min for 2 min, 10ml of supernatant was passed at the same velocity to bind fusion protein to protein A. After 20mM of sodium phosphate (pH 7.0) was passed at the same velocity for 2 min to wash, 500μl of the extracts were serially fractionated in a 1.5ml tube as 0.1M of citric acid (pH 3.0, Sigma, USA) was

passed at the the same velocity for 3 min. This was adjusted to pH 7.0 using 1M of Tris (pH 11.0, USB, USA), the existence of fusion proteins in tube was confirmed through ELISA as described above. The purified proteins were concentrated by centrifugation at 2000Xg, 4℃ for 30min using Centricon 30 (Amicon, USA)

Example 5.

SDS-PAGE of purified TNFR1-TNFR1/Fc and TNFR2-TNFR2/Fc (Fig. 15)

Proteins purified using protein A column were electrophorized by the method of SDS-PAGE in reducing condition added by DTT, reducing reagent (which destroy disulfide bond), and in a non-reducing condition excluding DTT. The result of the estimation of molecular weight on SDS-PAGE is shown in Table 10. It was possible to confirm that TNFR/Fc proteins were the shape of a dimer in the cell. The molecular weight deduced from the amino acid sequence of TNFR1-TNFR1-Ig was about 70kDa, and was estimated as about 102kDa on SDS-PAGE. As this difference could be regarded as a general phenomenon which generate on the electrophoresis of glycoproteins, this feature seemed to occurr as the result from decrease in mobility on the electrophoresis by the site of glycosylation.

Table 10. Molecular weight of TNFR-TNFR/Fc on the SDS-PAGE.

Proteins	Molecular weight (kDa)	
	Reducing condition	Non-reducing condition
TNFR1-TNFR1/Fc	102	200
TNFR2-TNFR2/Fc	115	220

Example 6.

Experiment of neutralization effect of simple/concatameric fusion dimeric TNFR/Fc fusion proteins on the cytotoxicity of TNFα and TNFβ

An L929 cell [ATCC, Mus musculus (mouse), NCTC clone 929 (derivative of strain L; L-929; L cell) was used for testing the effect of TNFR/Fc fusion protein on the inhibition of cytotoxicity induced by TNFα and TNFβ. This analysis was based on the TNFR activity of inhibiting cytotoxicity induced by TNF (Scallon et al., Cytokine 7:759, 1995).

L929 cells were inoculated to be $3X10^4$ cells/well in 96-well plates, and incubated at 37℃ for 24 hrs in a CO_2 incubator. Subsequently, actinomycin D (Sigma, USA) was added to 3μg/ml, and cells were incubated for 16~18 hrs with TNFα and TNFβ in the concentration of expressing 100% cytotoxicity (0.5~2ng/ml), and with serially 10 times diluted TNFR sample. Then, the cells in the 96-well plate were stained by the staining reagent, crystal violet (Wako Pure Chemical Industries, Japan) and the activity of the cells was estimated by the degree of absorbance at 595 nm wavelength using a spectrophotometer (Bio-Rad, Model-550, Japan).

As shown in Table 11 represented by IC_{50} of each TNFR/Fc fusion protein, concatameric fusion proteins (TNFR1-TNFR1/Ig and TNFR2-TNFR2/Ig) have shown the higher inhibitory effect on the cytotoxicity induced by two kinds of TNF than simple dimeric fusion proteins (TNFR1/Ig and TNFR2/Ig). Also, as compared with the effects of existing simple fusion dimer and concatameric shaped TNFR/Fc fusion protein dimer of the present invention on the inhibition of cytotoxicity of TNFα (Fig. 16) and TNFβ (Fig. 17), it more clearly appeared that concatameric shaped TNFR/Fc fusion protein dimers of the present invention remarkably inhibited the TNFα and TNFβ cytotoxicity.

Table 11. IC_{50} of cytotoxicity inhibition

Fusion proteins	IC50 (ug/ml)

		TNFα treated	TNFβ treated
Simple dimer	$[TNFR1/Fc]_2$	63	129
	$[TNFR2/Fc]_2$	189	469
Concatameric dimer	$[TNFR1\text{-}TNFR1/Fc]_2$	9	20
	$[TNFR2\text{-}TNFR2/Fc]_2$	15	15

Example 7

Experiment of suppressive effect of simple/concatameric fusion dimeric CD2/Fc fusion protein and CTLA4/Fc fusion protein on the proliferation of active immune cell

WT100B1S, a cell line of B lymphocyte which was made by transfection of pyrexia patient's B lymphocyte with Ebstein-Barr virus was incubated in RPMI 1640 supplemented with 10% FBS to use as antigen presenting cell of T lymphocyte. After centrifuged at 2,000rpm for 2 min to precipitate, this cells were resuspended in RPMI 1640 supplemented with 10% FBS to make $5.0X10^5$ cells/ml, then irradiated by 3,000 rd of γ-ray.

T lymphocytes were isolated from blood of healthy adult using Ficoll-hypaque (Amersham, USA), then incubated RPMI 1640 supplemented with 10% FBS to $2.0X10^6$ cells/ml.

To perform primary Mixed Lymphocyte Reaction (MLR), each 15ml of WT100B1S and T lymphocyte were mixed in 150mm cell culture dish, and incubated for 3 days, then added by 15ml of RPMI 1640 supplemented with 10% FBS and incubated for 3 days further. After incubated for total 6 days, live T lymphocytes were purified using Ficoll-hypaque (Amersham, USA) as described above, and purified T lymphocytes were stored in liquid nitrogen after freezing it by using the media comprising 45% FBS, 45% RPMI 1640, and 10% DMSO.

After T lymphocytes which were reacted by primary MLR were thawed to perform secondary MLR, the cells were washed with RPMI 1640 media for 2 times and made to be $3.0X10^5$ cells/ml in RPMI 1640 supplemented with 10% FBS.

WT100B1S using as antigen presenting cell was newly cultured by the method as described above, then prepared by irradiation of 3,000 rd of γ-ray and to be $7.5X10^4$ cells/ml in RPMI 1640 supplemented with 10% FBS. After 100μl of prepared WT100B1S was added in 96-well flat bottom cell culture plate and mixed with CD2/Fc and CTLA4/Fc fusion protein at final concentration of 10, 1, 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}μg/ml, 100μl of primary MLR reacted T lymphocytes as above was added. After incubated for 2 days in 5% CO_2, 37℃ incubator, 100μl of RPMI 1640 supplemented with 10% FBS was added and incubated for 2 days further. In the last 6 hrs of the total 6 days culture, cells were incubated with addition of 1.2μCi/ml of ^{3}H-thymidine (Amersham, USA).

At the end of culturing, supernatants were removed after centrifugation of 96-well plate was performed at 4℃, 110Xg for 10 min to precipitate T lymphocytes, and pellets were washed with 200μl of 1XPBS. Centrifugation was performed in the same condition and PBS was removed, then 200μl of ice-cold trichloridic acid (TCA, Merck, USA) was added and mixed for 2 min, then reacted at 4℃ for 5 min to remove residue of ^{3}H-thymidine.

After centrifugation in the same condition as described above, supernatants were removed and T lymphocytes were fixed by incubation at 4℃ for 5 min after 200μl of ice-cold 70% ethanol was added. Supernatants were removed after centrifugation, and ^{3}H-thymidine (Amersham, USA) residue was completely removed by treatment of 10% TCA in the same method as described above.

Cell lysis was performed by reaction with 100μl of 2% SDS (pH 8.0) and 0.5N of NaOH at 37℃ for 30min, and T lymphocytes were precipitated by centrifugation at 25℃, 110Xg for 10min, and then 50μl of supernatants was transferred to 96-well sample plate (Wallac, USA). After 1.5 volume of OptiPhase SuperMix (Wallac, USA) was added into the supernatants and mixed for 5 min, the existence of T lymphocyte proliferation was confirmed by measurement of cpm value of ^{3}H using 1450 MicroBeta TriLux microplate liquid scintillation andluminescence counter (Wallac, USA).

Example 8

Experiment of effect on increase of plasma half-life of glycosylated concatameric fusion dimeric proteins in mouse

The measurement of plasma half-life of glycosylated concatameric fusion dimeric proteins, [mgTNFR1-TNFR1/Fc]2, [mgTNFR2-TNFR2/Fc]2, [mgCD2-CD2/Fc]2, and [mgCTLA4-CTLA4/Fc]2 was performed by measuring the concentration of proteins using ELISA after 5μg of purified fusion proteins was i.p. injected into mouse (ICR, Samtako, Korea) and bloods were extracted at regular interval for 120 hrs (5 days) as maximum. As shown Fig. 20, Fig. 21, and Fig 22, it could be seen that the plasma half-life of glycosylated concatameric fusion dimeric proteins have been increased in comparison of the corresponding simple fusion dimeric proteins of native shape, and the increase in efficacy through continuous effect could be expected.

Example 9

Experiment of effects of simple/concatameric TNFR/Fc fusion protein dimers on collagen-induced arthritis of DBA/1 mouse

Collagen Induced Arthritis (CIA) was developed by injection with 100μg per DBA/1 mouse of type II collagen dissolved at 2mg/ml concentration in 0.05M acetic acid and Arthrogen-CIA adjuvant (Chondrex, USA) into tail. Boosting was performed after 3 weeks, and incomplete Freund's adjuvant (Difco, USA) was used.

Arthritis was developed 3~4 weeks after immunization with 100μg of type II collagen in the DBA/1 mice. Red and swollen paws of mice had been observed 3~5 days after onset, and inflammatory arthritis lasted more than 3 - 4 weeks. Although inflammation was eventually alleviated, damaged joints remained rigid permanently. The degree of

arthritis was measured 2~3 times per week on the basis of table 12 which represented subjective index of arthritis severity (measure average of five mice in each experiment). To measure the effects of simple and concatameric fusion dimeric TNFR/Fc on CIA, TNFR/Fc or PBS was i.p. injected into the mice. TNFR/Fc was injected with 10μg at every 2 days for 19~45 days into 5 mice per experiments (arrows in Fig. 23). PBS was injected into 5 mice as control. As shown in Fig. 7, in the case of mice injected with existing simple dimeric shaped TNFR/Fc fusion protein, it could be seen that the effect decreased to about 26-38% in comparison with the figures of arthritis index in mice injected with PBS as control, but 42-55% decreased in case of concatameric shaped dimer, $[TNFR1\text{-}TNFR1/Fc]_2$ and $[TNFR2\text{-}TNFR2/Fc]_2$ were injected. Therefore, it could be shown that concatameric fusion dimeric TNFR/Fc fusion proteins have remarkably decreased arthritis of mouse than existing simple fusion dimeric TNFR/Fc fusion proteins.

Table 12. Severity score of arthritis

Severity score	Condition of disease
0	No erythema and swelling
1	Erythema and mild swelling limited to ankle and tarsal
2	Erythema and mild swelling spread from ankle to tarsal
3	Erythema and mild swelling spread from ankle to metatarsal joint
4	Erythema and severe swelling expend to ankle, legs, and digits

The results as above represented that concatameric shaped dimeric TNFR/Fc fusion proteins were more effective in decreasing the rate of CIA development than existing simple dimeric fusion proteins, therefore, as use in arthritis therapy, concatameric shaped protein compositions could be more effective therapeutics than existing protein compositions.

The concatameric proteins, concatameric fusion dimeric proteins and their glycosylated proteins of the present invention were able to express increased efficacy and high stability, and to be produced with high yield.

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Korea Cell Line Research Foundation(KCLRF)

Address of depositary institution(*including postal code and country*)

Cancer Research Institute, Seoul National University College of Medicine
28 Yongon-dong, Chongno-gu
SEOUL 120-091 Republic of Korea

Date of deposit	Accession Number
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For receiving Office use only	For international Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

Form PCT/RO/134(July 1998)

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 29, line 15-20
B. IDENTIFICATION OF DEPOSIT **Further deposits are on an additional sheet** ☐
Name of depositary institution **Korean Culture Center of Microorganisms(KCCM)**
Address of depositary institution(*including postal code and country*) **361-221, Yurim B/D, Hongje-1-dong, Seodaemun-gu,** **SEOUL 120-091, Republic of Korea**
Date of deposit **11/07/2002** | **Accession Number** **KCCM 10403**
C.ADDITIONAL INDICATIONS *(leave blank if not applicable)* **This information is continued on an additional sheet** ☐
D.DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*
E.SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*
The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For international Bureau use only
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Authorized officer	Authorized officer

5 Form PCT/RO/134(July 1998)

INDICATIONS RELATING TO DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 29, line 15-20
B. IDENTIFICATION OF DEPOSIT Further deposits are on an additional sheet☐
Name of depositary institution Korean Culture Center of Microorganisms(KCCM)
Address of depositary institution(*including postal code and country*) 361-221, Yurim B/D, Hongje-1-dong, Seodaemun-gu, SEOUL 120-091, Republic of Korea

Date of deposit	Accession Number
11/07/2002	KCCM 10405

C.ADDITIONAL INDICATIONS(*leave blank if not applicable*) This information is continued on an additional sheet ☐
D.DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE(*if the indications are not for all designated States*)
E.SEPARATE FURNISHING OF INDICATIONS(*leave blank if not applicable*)
The indications listed below will be submitted to the International Bureau later(*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

For receiving Office use only	For international Bureau use only
☐ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer	Authorized officer

5 Form PCT/RO/134(July 1998)

WHAT IS CLAIMED IS:

1. A concatameric protein comprising two soluble domains, in which a N-terminus of a soluble domain of a biologically active protein is linked to C- terminus of an identical soluble domain or a different soluble domain of a biologically active protein.

2. A concatameric fusion dimeric protein comprising two monomeric proteins formed by linkage of a concatamer of two identical soluble extracellular domains of proteins involving immune response to a hinge region of an Fc fragment of an immunoglobulin molecule, wherein said monomeric proteins are linked by intermolecular disulfide bonds at the hinge region, and having improved stability and therapeutic effects.

3. The concatameric fusion dimeric protein as set forth in claim 2, wherein the immunoglobulin molecule is IgG.

4. The concatameric fusion dimeric protein as set forth in claim 2, wherein the protein involving immune response is selected from the group consisting of cytokines, cytokine receptors, adhesion molecules, tumor necrosis factor receptors, receptor tyrosine kinases, chemokine receptors and other cell surface proteins which contain a soluble extracellular domain.

5. The concatameric fusion dimeric protein as set forth in claim 4, wherein the protein is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-17, TNF, TGF, IFN, GM-CSF, G-CSF, EPO, TPO, M-CSF, GHR, IL-13R, IL-1R, IL-2R, IL-3R, IL-4R, IL-5R, IL-6R, IL-7R, IL-9R, IL-15R, TNFR, TGFR, IFNR, interferon-α R, -β R and -γ R, GM-CSFR, G-CSFR, EPOR, cMpl, gp130, Fas (Apo 1), CCR1, CXCR1-4, TrkA, TrkB, TrkC, Htk, REK7, Rse/Tyro-3, hepatocyte growth factor R, platelet-derived growth factor R, Flt-1, CD2, CD4, CD5, CD6, CD22, CD27, CD28, CD30, CD31, CD40, CD44, CD100, CD137, CD150, LAG-3, B7, B61, β-neurexin, CTLA-4, ICOS, ICAM-1, complement R-2 (CD21), IgER, lysosomal membrane gp-1, α2-microglobulin receptor-related proteins, and sodium-releasing peptide R.

6. The concatameric fusion dimeric protein as set forth in claim 2, wherein the monomeric protein contains an amino acid sequence of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 18, or SEQ ID NO: 20.

7. A DNA construct encoding a monomeric protein formed by linkage of a concatamer of two identical soluble extracellular domains of a protein involving immune response to a hinge region of an Fc fragment of an immunoglobulin molecule.

8. The DNA construct as set forth in claim 7, wherein the immunoglobulin molecule is IgG.

9. The DNA construct as set forth in claim 7, wherein the protein involving immune response is selected from the group consisting of cytokines, cytokine receptors, adhesion molecules, tumor necrosis factor receptors, receptor tyrosine kinases, chemokine receptors and other cell surface proteins which contain a soluble extracellular domain.

10. The DNA construct as set forth in claim 9, wherein the protein is selected from the group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-17, TNF, TGF, IFN, GM-CSF, G-CSF, EPO, TPO, M-CSF, GHR, IL-13R, IL-1R, IL-2R, IL-3R, IL-4R, IL-5R, IL-6R, IL-7R, IL-9R, IL-15R, TNFR, TGFR, IFNR, interferon-α R, -β R and -γ R, GM-CSFR, G-CSFR, EPOR, cMpl, gp130, Fas (Apo 1), CCR1, CXCR1-4, TrkA, TrkB, TrkC, Htk, REK7, Rse/Tyro-3, hepatocyte growth factor R, platelet-derived growth factor R, Flt-1, CD2, CD4, CD5, CD6, CD22, CD27, CD28, CD30, CD31, CD40, CD44, CD100, CD137, CD150, LAG-3, B7, B61, β-neurexin, CTLA-4, ICOS, ICAM-1, complement R-2 (CD21), IgER, lysosomal membrane gp-1, α2-microglobulin receptor-related proteins, and sodium-releasing peptide R.

11. The DNA construct as set forth in claim 7, wherein the DNA construct contains a nucleotide sequence of SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 17, or SEQ ID NO: 19.

12. A recombinant expression plasmid comprising the DNA construct of claim 7 operably linked thereto.

13. The recombinant expression plasmid as set forth in claim 12, wherein the recombinant expression plasmid is a pTR11-Top10' plasmid (accession No.: KCCM 10288), a pTR22-Top10' plasmid (accession No.: KCCM 10289), a pCD22Ig plasmid (accession No.: KCCM 10402), or a pCT44Ig plasmid (accession No.: KCCM 10400).

14. A host cell transformed or transfected with the recombinant expression plasmid of claim 12.

15. The host cell as set forth in claim 14, wherein the host cell is a mammalian cell.

16. The host cell as set forth in claim 14 or 15, wherein the recombinant expression plasmid is a pTR11-Top10' plasmid (accession No.: KCCM 10288), a pTR22-Top10' plasmid (accession No.: KCCM 10289), a pCD22Ig plasmid (accession No.: KCCM 10402), or a pCT44Ig plasmid (accession No.: KCCM 10400).

17. The host cell as set forth in claim 16, wherein the host cell is a TR11Ig-CHO cell line (accession No.: KCLRF-BP-00046) or a TR22Ig-CHO cell line (accession No.: KCLRF-BP-00049).

18. A method of preparing a concatameric fusion dimeric protein in which disulfide bonds are formed between the hinge regions of two monomeric proteins, comprising the steps of:

culturing the transformed or transfected host cell of claim 14 under conditions suitable for expression of a DNA construct encoding a concatameric fusion monomeric protein in which a concatamer of two identical soluble extracellular domains of

proteins involving immune response is linked to a hinge region of an Fc fragment of an immunoglobulin molecule; and

isolating and purifying a dimeric protein formed by dimerization of the produced monomeric proteins from culture medium.

19. The method as set forth in claim 18, wherein the DNA construct encoding a concatameric fusion monomeric protein is prepared by preparing a DNA construct encoding a simple fusion monomeric protein formed by joining a DNA fragment encoding an Fc fragment of an immunoglobulin molecule and a DNA fragment encoding a soluble extracellular domain of a protein involving immune response; and joining the prepared DNA construct and a second DNA fragment identical to the DNA fragment encoding a soluble extracellular domain of a protein involving immune response.

20. The method as set forth in claim 19, wherein the DNA construct encoding a concatameric fusion monomeric protein contains a glycosylation motif sequence.

21. The method as set forth in claim 20, wherein the glycosylation motif sequence is inserted to a region at which two soluble extracellular domains are joined.

22. The method as set forth in claim 19, wherein the concatameric fusion monomeric protein contains a leader sequence.

23. The method as set forth in claim 22, wherein the concatameric fusion monomeric protein is CTLA-4, and the leader sequence has an amino acid sequence of MACLGFQRHKAQKNLAARTWPCTLLFFIPVFCKA.

24. The method as set forth in claim 23, wherein the leader sequence has an amino acid sequence of MRTWPCTLLFFIPVFCKA excluding ACLGFQRHKAQKNLAA.

25. The method as set forth in any of claims 18 to 24, wherein the host cell is a mammalian cell.

26. A concatameric fusion dimeric protein comprising two monomeric proteins formed by linkage of a concatamer of two identical soluble extracellular domains of proteins involving immune response to the hinge region of Fc fragment of an immunoglobulin molecule, wherein said monomeric proteins are linked by formation of intermolecular disulfide bonds at the hinge region and glycosylated, and having improved stability and therapeutic effects.

27. The concatameric fusion dimeric protein as set forth in claim 26, wherein the monomeric protein contains an amino acid sequence of SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 22, or SEQ ID NO: 24.

28. A DNA construct encoding a monomeric protein formed by linkage of a concatamer of two identical soluble extracellular domains of proteins involving immune response to a hinge region of an Fc fragment of an immunoglobulin molecule and containing glycosylation motif peptides.

29. The DNA construct as set forth in claim 28, wherein the DNA construct contains an amino acid sequence of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 21, or SEQ ID NO: 23.

30. A recombinant expression plasmid operably linked to the DNA construct of claim 28.

31. The recombinant expression plasmid as set forth in claim 30, wherein the recombinant expression plasmid is a pTR11Ig-MG plasmid (accession No.: KCCM 10404), a pTR22Ig-MG plasmid (accession No.: KCCM 10407), a pCD22Ig-MG plasmid (accession No.: KCCM 10401), or a pCT44Ig-MG plasmid (accession No.: KCCM 10399).

32. A host cell transformed or transfected with the recombinant expression plasmid of claim 30.

33. The host cell as set forth in claim 32, wherein the host cell is a mammalian cell.

34. A pharmaceutical or diagnostic composition comprising the dimeric protein of claim 2.

35. A pharmaceutical or diagnostic composition comprising the glycosylated dimeric protein of claim 26.

FIG. 1

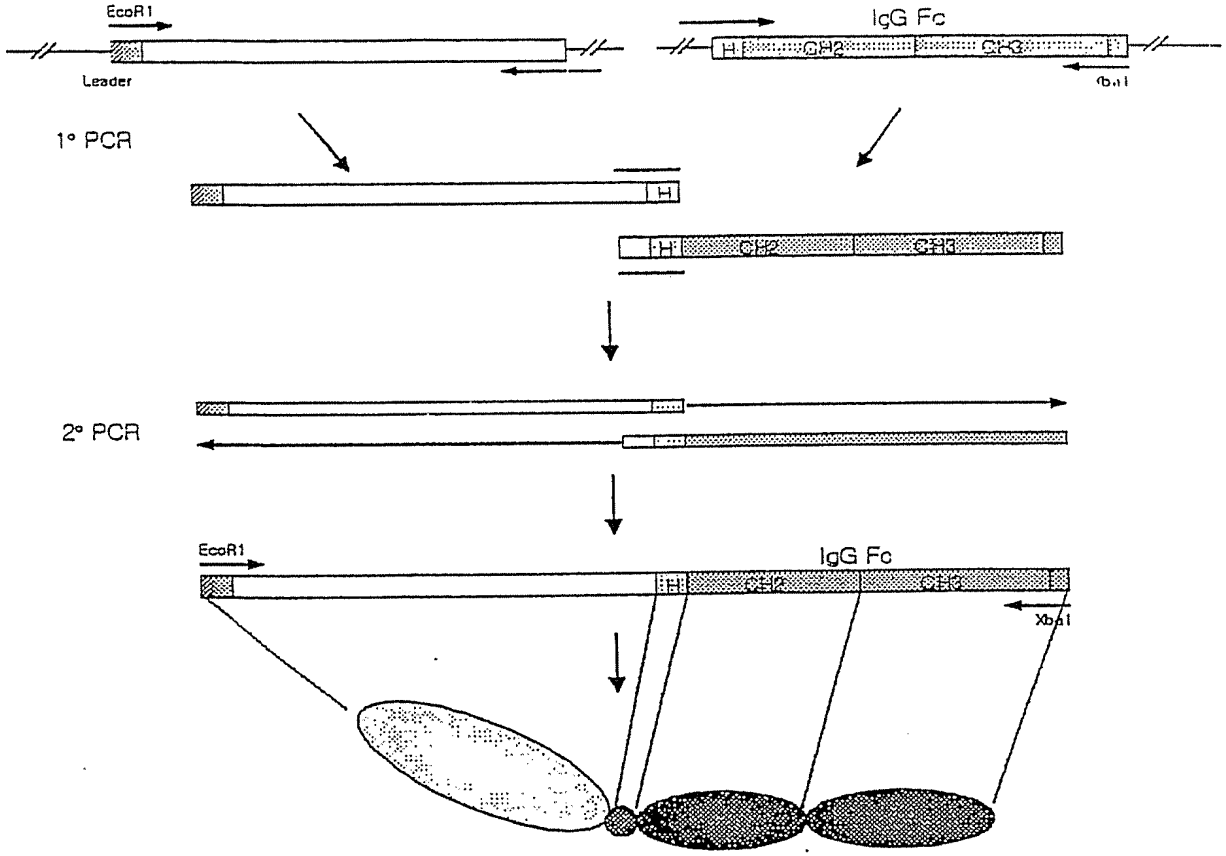


FIG. 2

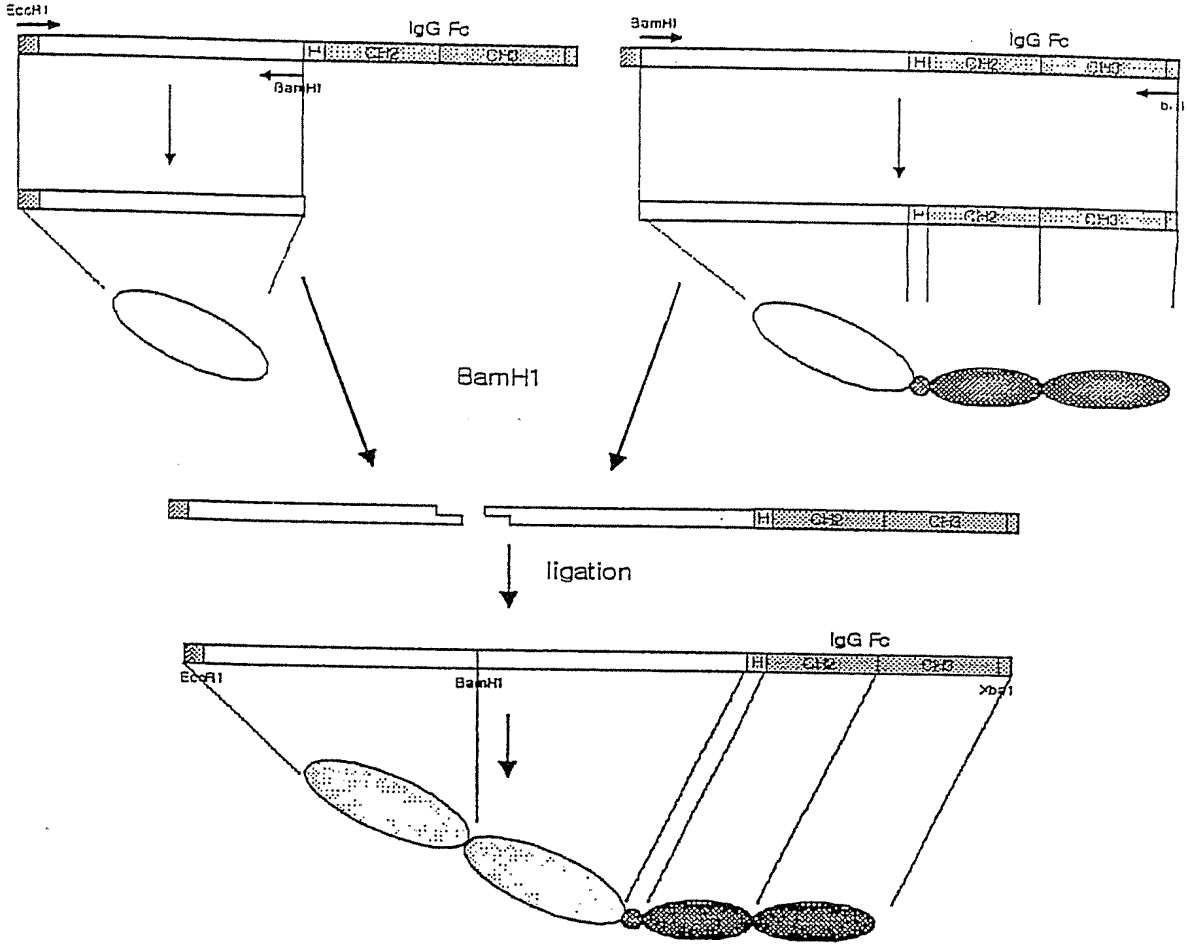

EcoR1
IgG Fc
H
CH2
CH3
BamH1
BamH1
IgG Fc
BamH1
ligation
IgG Fc
EcoR1
BamH1
Xba1

FIG. 3

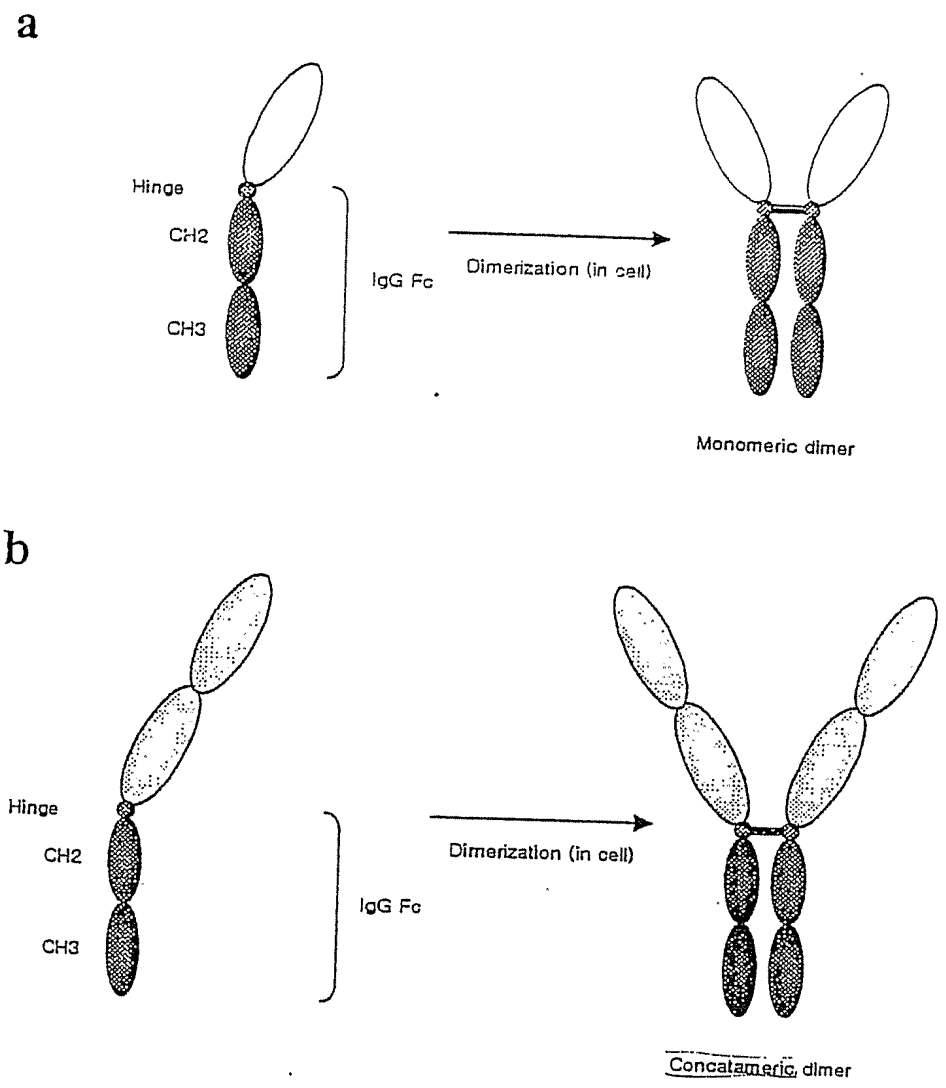

a
Hinge
CH2
CH3
IgG Fc
Dimerization (in cell)
Monomeric dimer
b
Hinge
CH2
CH3
IgG Fc
Dimerization (in cell)
Concatameric dimer

FIG. 4

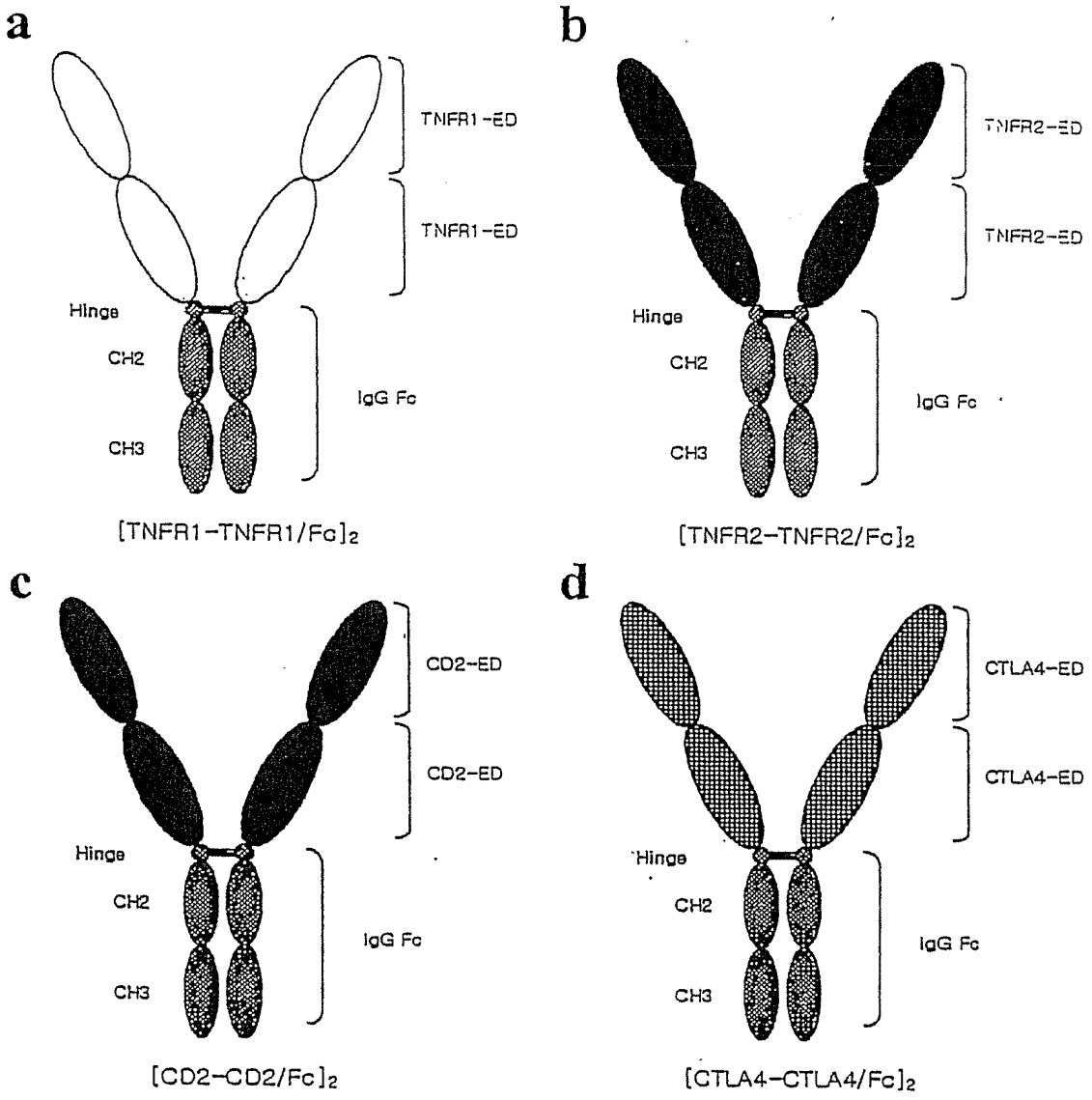

a
TNFR1-ED
TNFR1-ED
Hinge
CH2
CH3
IgG Fc
[TNFR1-TNFR1/Fc]2
b
TNFR2-ED
TNFR2-ED
Hinge
CH2
CH3
IgG Fc
[TNFR2-TNFR2/Fc]2
c
CD2-ED
CD2-ED
Hinge
CH2
CH3
IgG Fc
[CD2-CD2/Fc]2
d
CTLA4-ED
CTLA4-ED
Hinge
CH2
CH3
IgG Fc
[CTLA4-CTLA4/Fc]2

FIG. 5

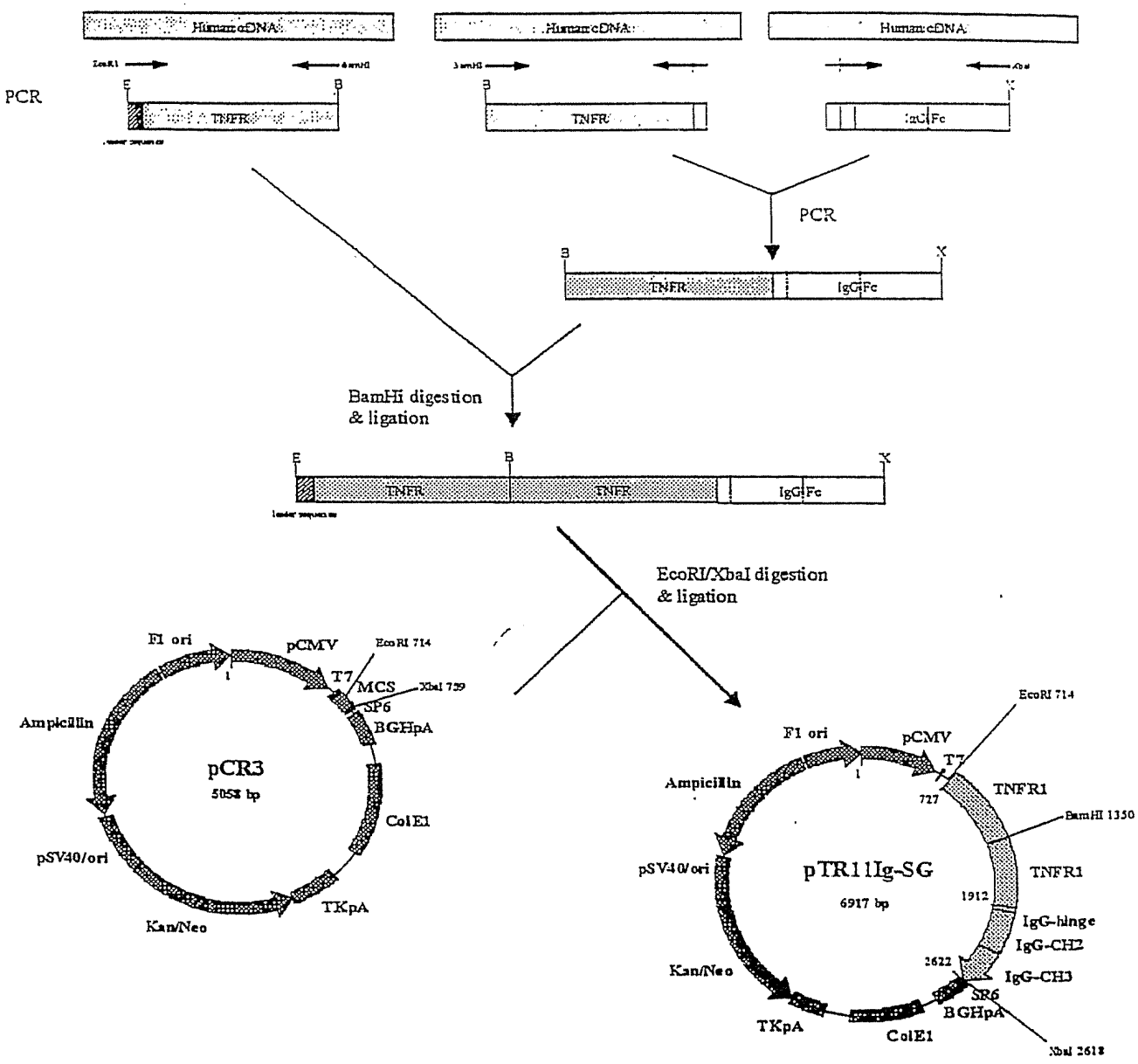

Human cDNA
Human cDNA
Human cDNA
PCR
TNFR
TNFR
IgG Fc
PCR
TNFR
IgG Fc
BamHi digestion
& ligation
TNFR
TNFR
IgG Fc
EcoRI/XbaI digestion
& ligation
F1 ori
pCMV
EcoRI 714
T7
MCS
XbaI 729
SP6
BGHpA
Ampicillin
pCR3
5058 bp
ColE1
pSV40/ori
TKpA
Kan/Neo
EcoRI 714
F1 ori
pCMV
T7
TNFR1
727
BamHI 1350
Ampicillin
pSV40/ori
pTR11Ig-SG
6917 bp
TNFR1
1912
IgG-hinge
IgG-CH2
2622
IgG-CH3
Kan/Neo
SP6
BGHpA
TKpA
ColE1
XbaI 2618

FIG. 6

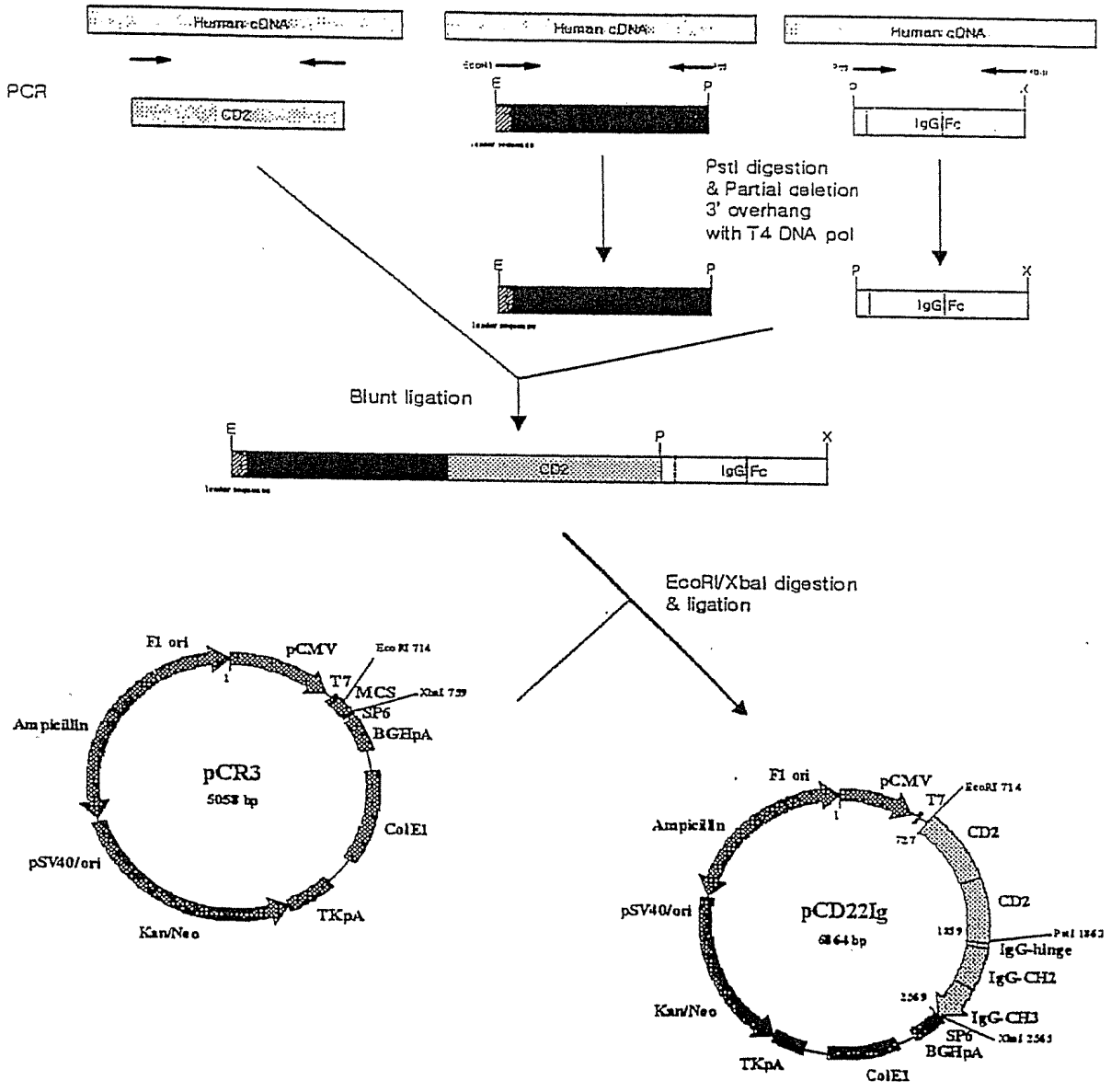

Human cDNA
Human cDNA
Human cDNA
PCR
CD2
IgG Fc
PstI digestion
& Partial deletion
3' overhang
with T4 DNA pol
Blunt ligation
CD2
IgG Fc
EcoRI/XbaI digestion
& ligation
pCR3
5053 bp
F1 ori
pCMV
T7
MCS
SP6
BGHpA
ColE1
TKpA
Kan/Neo
pSV40/ori
Ampicillin
pCD22Ig
6864 bp
CD2
IgG-hinge
IgG-CH2
IgG-CH3

FIG. 7

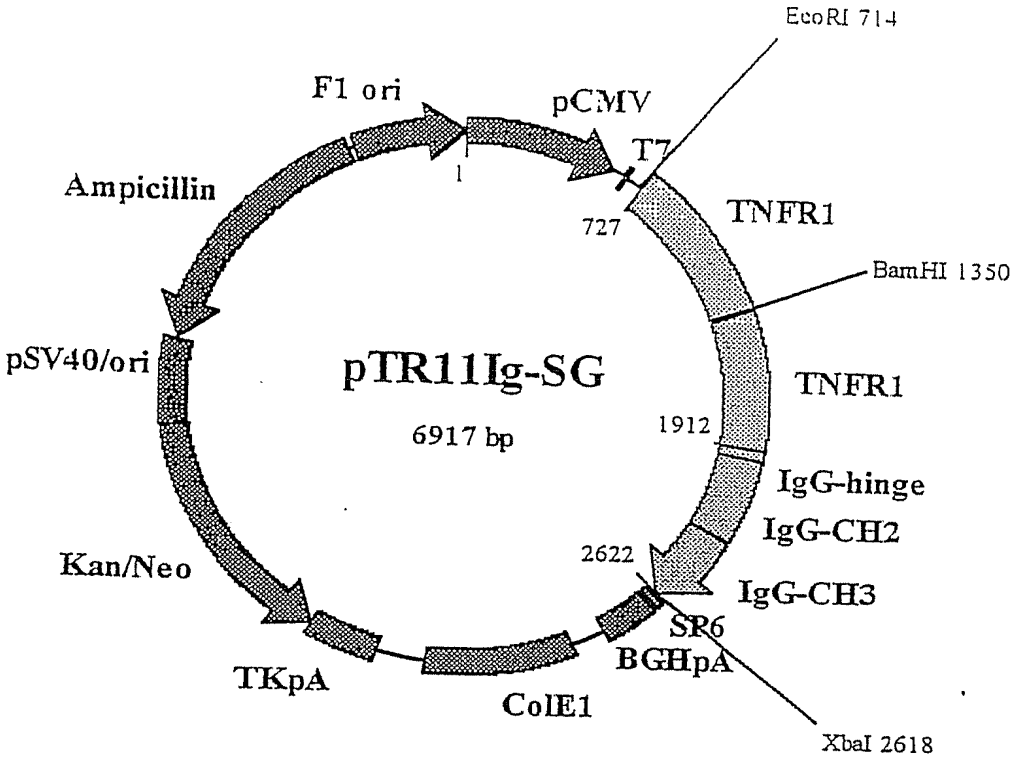

EcoRI 714
F1 ori
pCMV
T7
1
Ampicillin
727
TNFR1
BamHI 1350
pSV40/ori
pTR11Ig-SG
6917 bp
TNFR1
1912
IgG-hinge
IgG-CH2
2622
Kan/Neo
IgG-CH3
SP6
BGHpA
TKpA
ColE1
XbaI 2618

FIG. 8

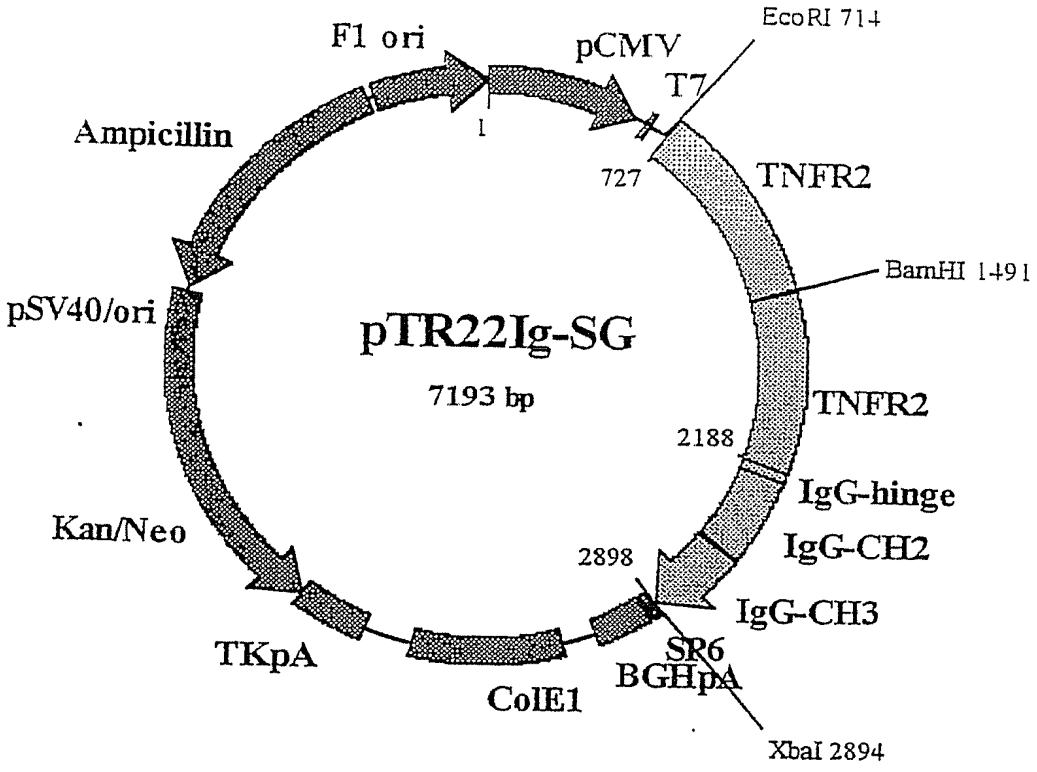

F1 ori
pCMV
EcoRI 714
T7
1
727
TNFR2
Ampicillin
BamHI 1491
pSV40/ori
pTR22Ig-SG
7193 bp
TNFR2
2188
IgG-hinge
IgG-CH2
Kan/Neo
2898
IgG-CH3
SP6
TKpA
BGHpA
ColE1
XbaI 2894

FIG. 9

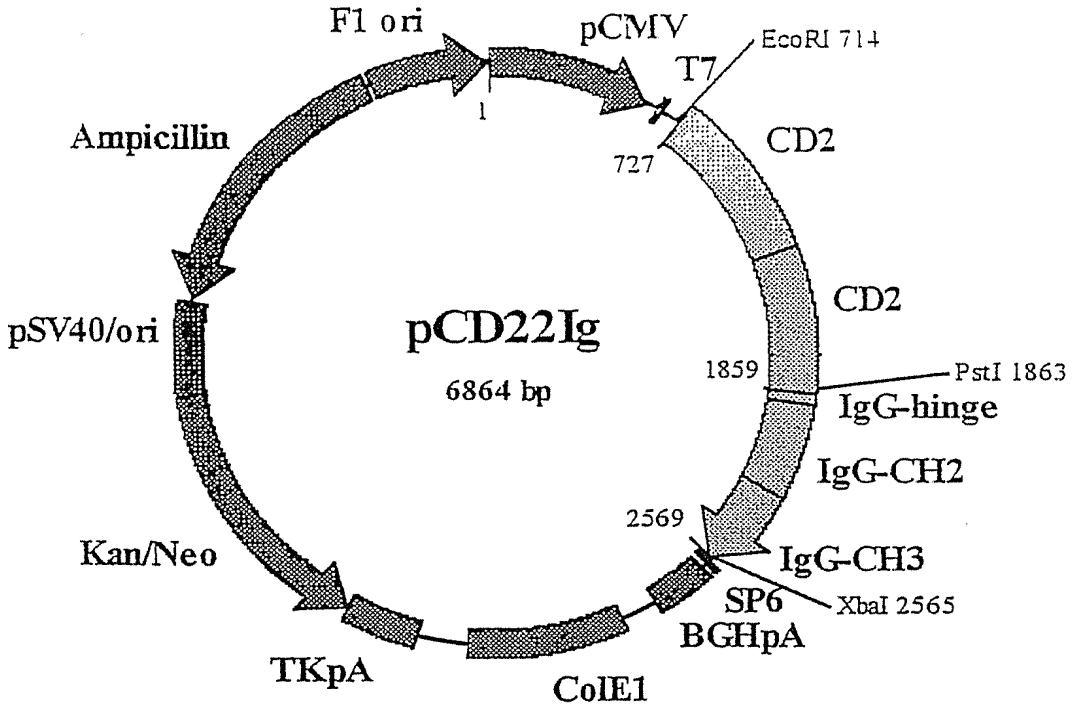

F1 ori
pCMV
T7
EcoRI 714
1
Ampicillin
727
CD2
CD2
pSV40/ori
pCD22Ig
6864 bp
1859
PstI 1863
IgG-hinge
IgG-CH2
2569
IgG-CH3
Kan/Neo
SP6
XbaI 2565
BGHpA
TKpA
ColE1

FIG. 10

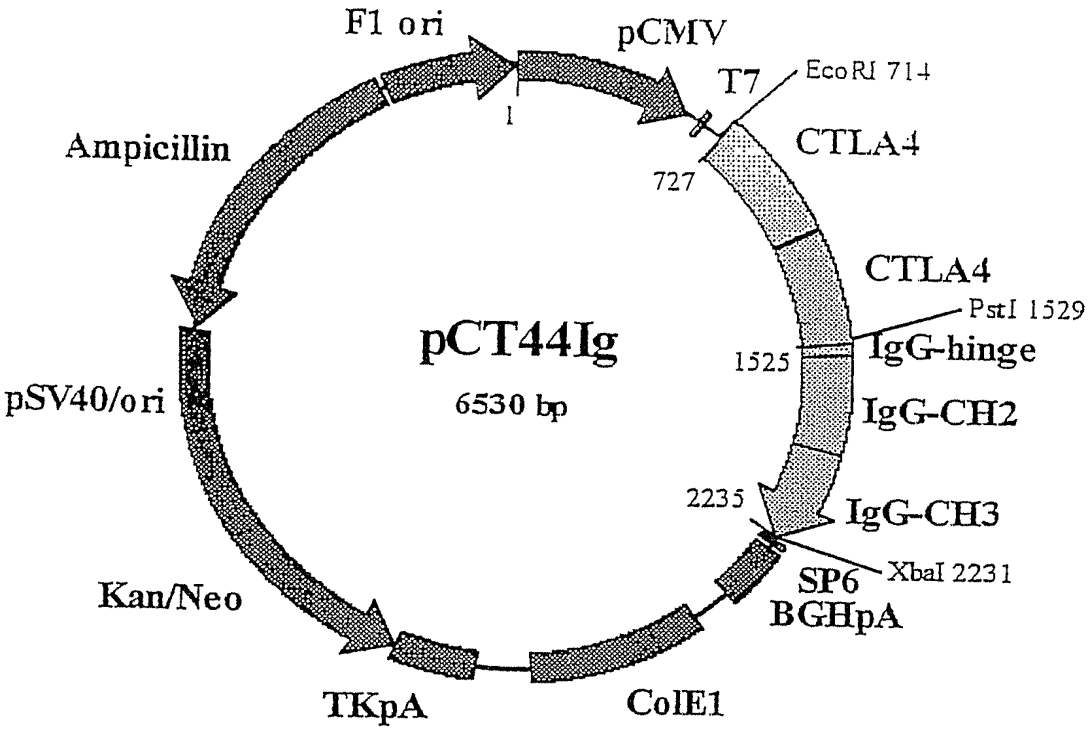

F1 ori
pCMV
T7
EcoRI 714
1
Ampicillin
CTLA4
727
CTLA4
PstI 1529
pCT44Ig
6530 bp
1525
IgG-hinge
pSV40/ori
IgG-CH2
2235
IgG-CH3
XbaI 2231
SP6
BGHpA
Kan/Neo
TKpA
ColE1

FIG. 11

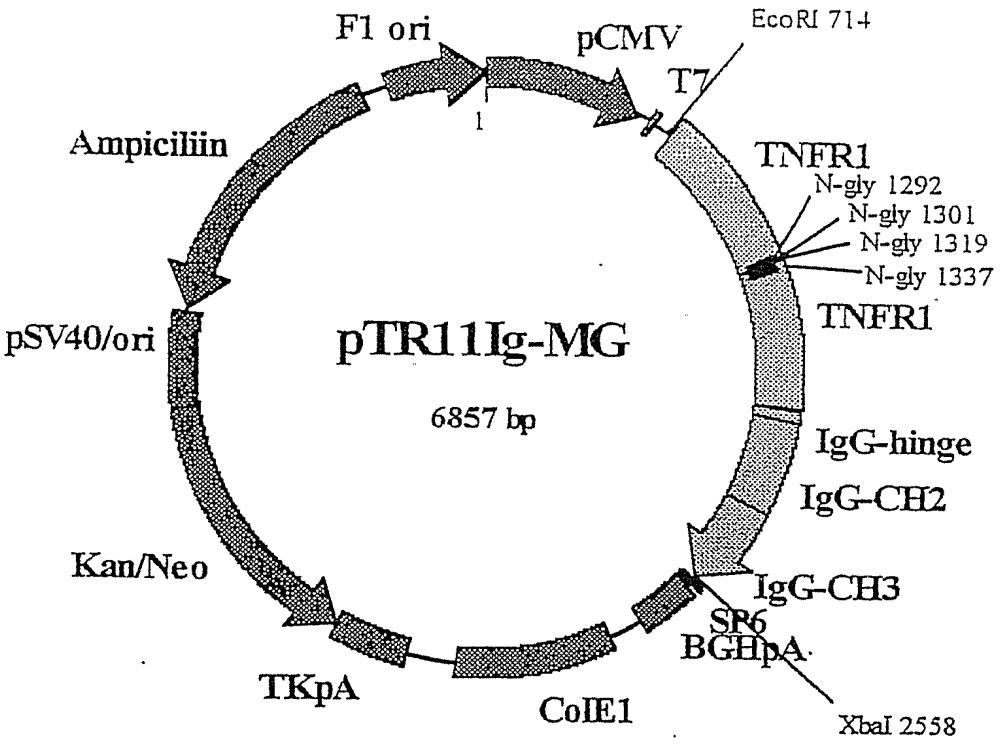

F1 ori
pCMV
EcoRI 714
T7
1
Ampiciliin
TNFR1
N-gly 1292
N-gly 1301
N-gly 1319
N-gly 1337
TNFR1
pSV40/ori
pTR11Ig-MG
6857 bp
IgG-hinge
IgG-CH2
Kan/Neo
IgG-CH3
SP6
BGHpA
TKpA
ColE1
XbaI 2558

FIG. 12

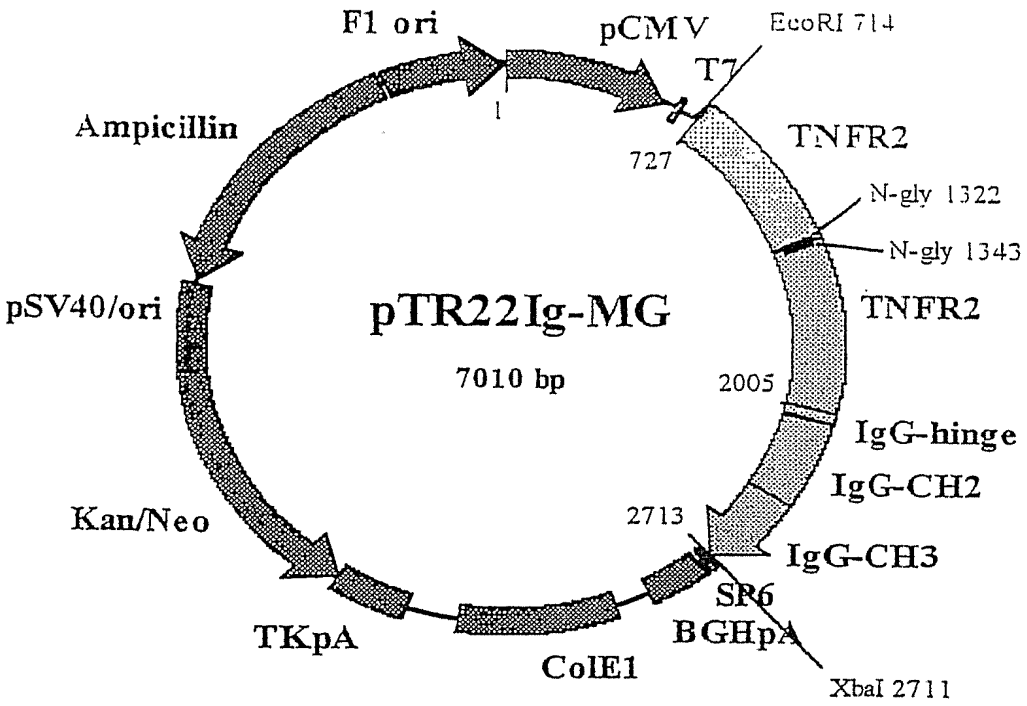

F1 ori
pCMV
EcoRI 714
T7
1
Ampicillin
TNFR2
727
N-gly 1322
N-gly 1343
pSV40/ori
pTR22Ig-MG
7010 bp
TNFR2
2005
IgG-hinge
IgG-CH2
Kan/Neo
2713
IgG-CH3
SP6
BGHpA
TKpA
ColE1
XbaI 2711

FIG. 13

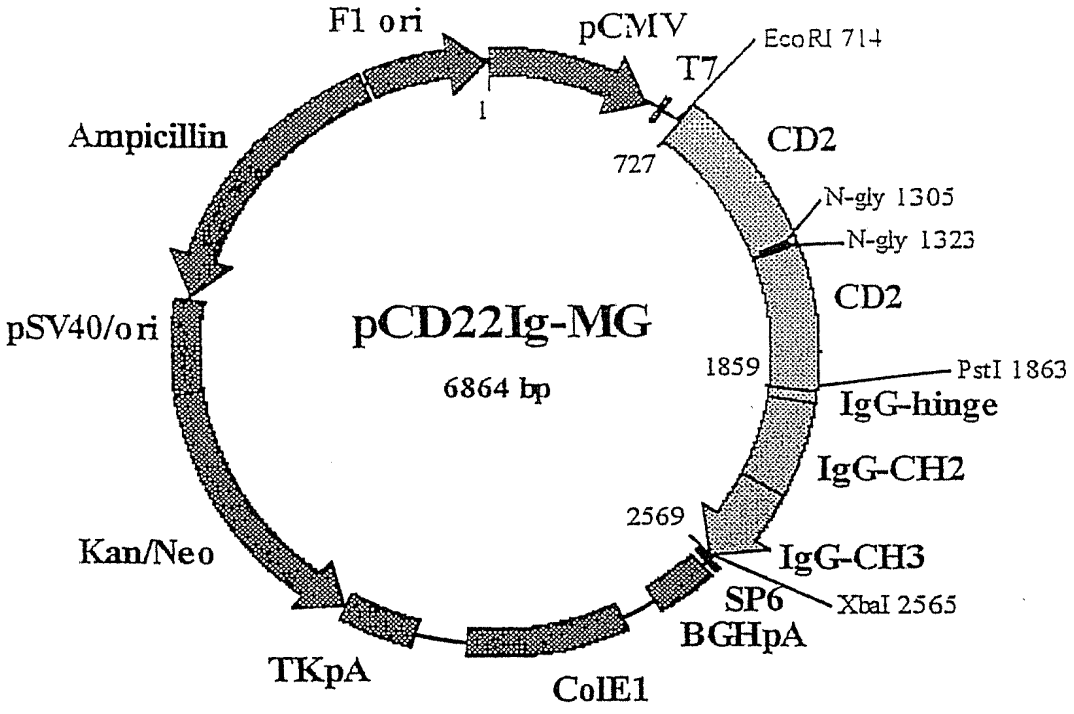

F1 ori
pCMV
EcoRI 714
T7
1
727
CD2
Ampicillin
N-gly 1305
N-gly 1323
CD2
pSV40/ori
pCD22Ig-MG
6864 bp
1859
PstI 1863
IgG-hinge
IgG-CH2
2569
IgG-CH3
Kan/Neo
SP6
XbaI 2565
BGHpA
TKpA
ColE1

FIG. 14

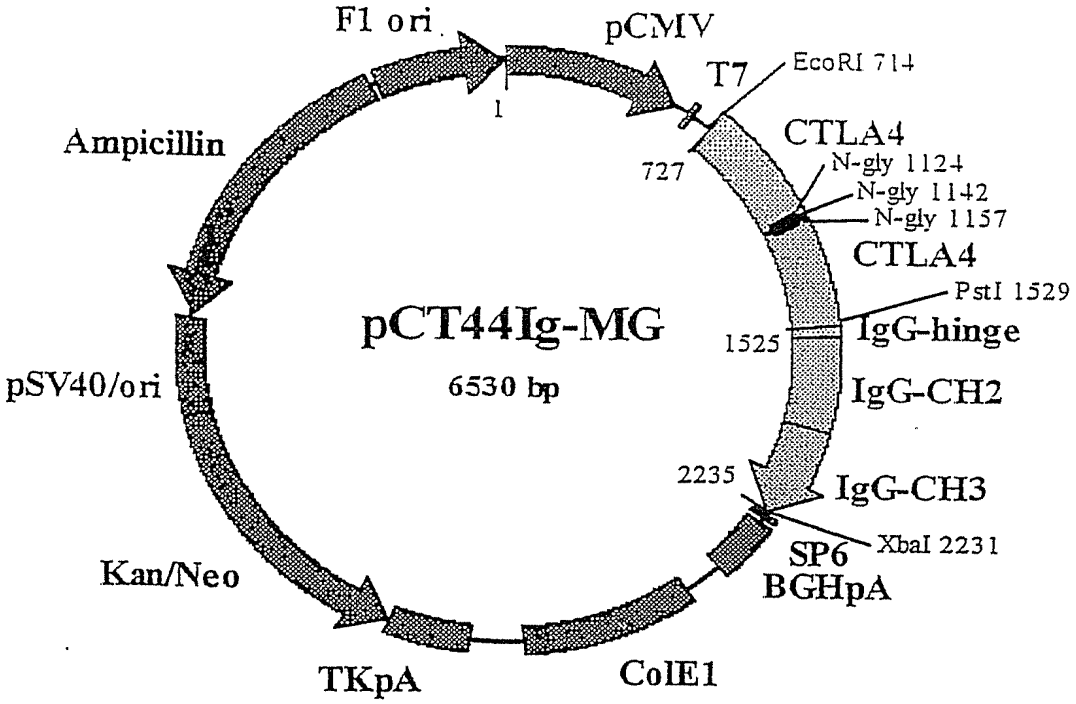

F1 ori
pCMV
T7
EcoRI 714
1
CTLA4
727
N-gly 1124
N-gly 1142
N-gly 1157
CTLA4
PstI 1529
Ampicillin
pCT44Ig-MG
6530 bp
1525
IgG-hinge
pSV40/ori
IgG-CH2
2235
IgG-CH3
XbaI 2231
SP6
BGHpA
Kan/Neo
TKpA
ColE1

FIG. 15

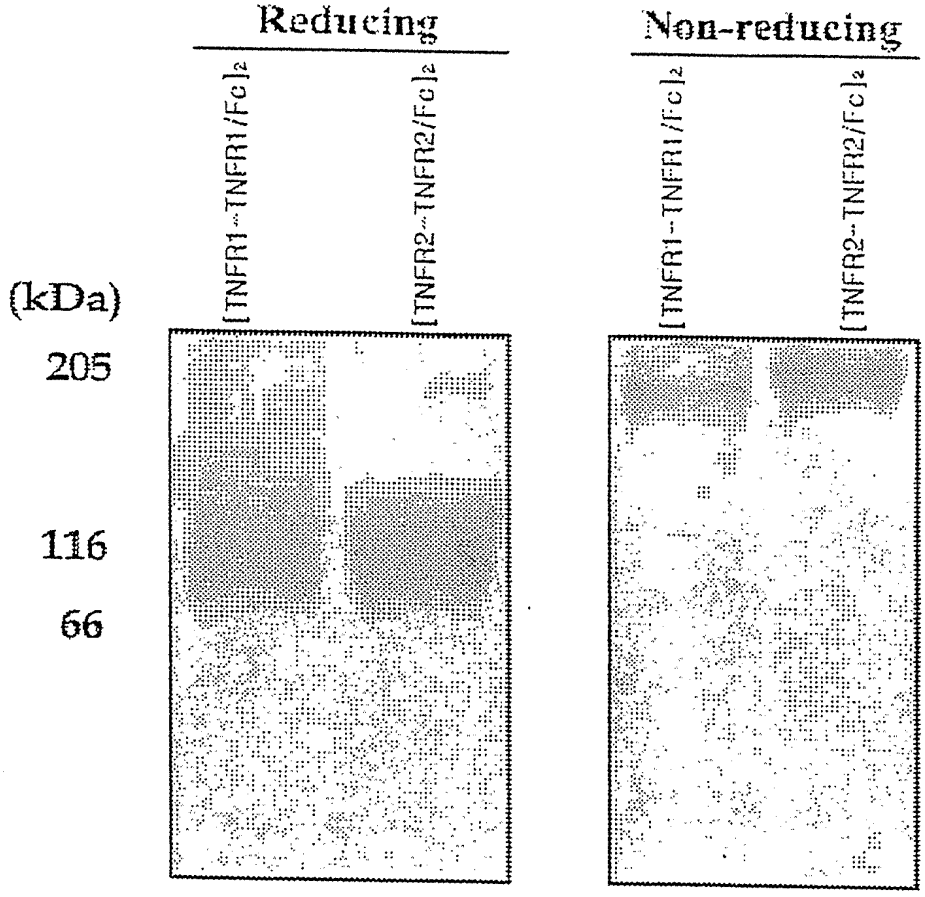

Reducing
Non-reducing
[TNFR1-TNFR1/Fc]2
[TNFR2-TNFR2/Fc]2
[TNFR1-TNFR1/Fc]2
[TNFR2-TNFR2/Fc]2
(kDa)
205
116
66

FIG. 16

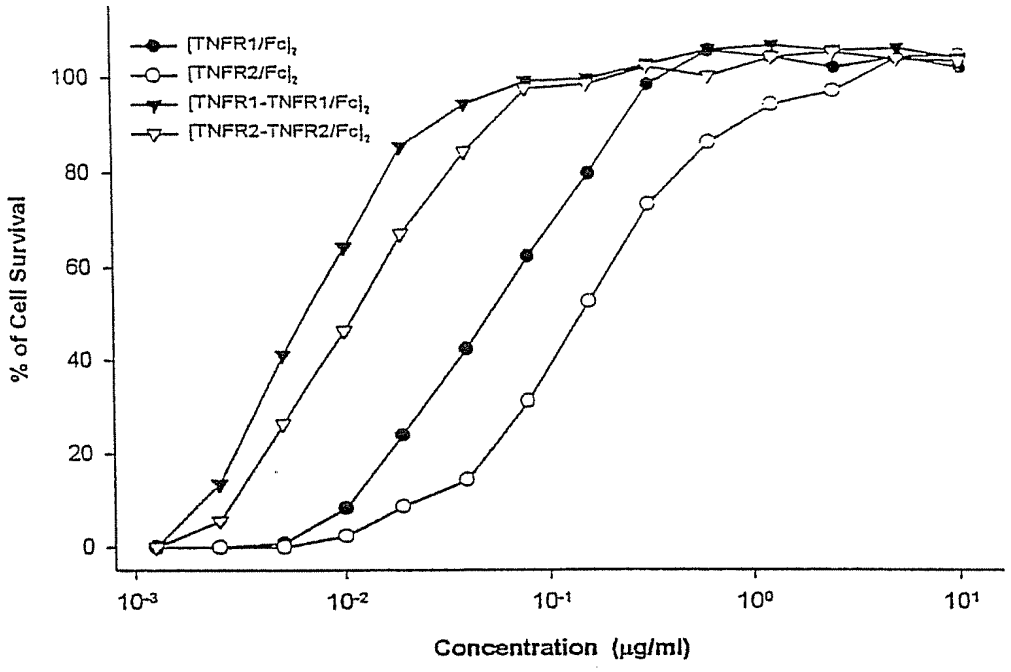

[TNFR1/Fc]2
[TNFR2/Fc]2
[TNFR1-TNFR1/Fc]2
[TNFR2-TNFR2/Fc]2
% of Cell Survival
100
80
60
40
20
0
10-3
10-2
10-1
100
101
Concentration (μg/ml)

FIG. 17

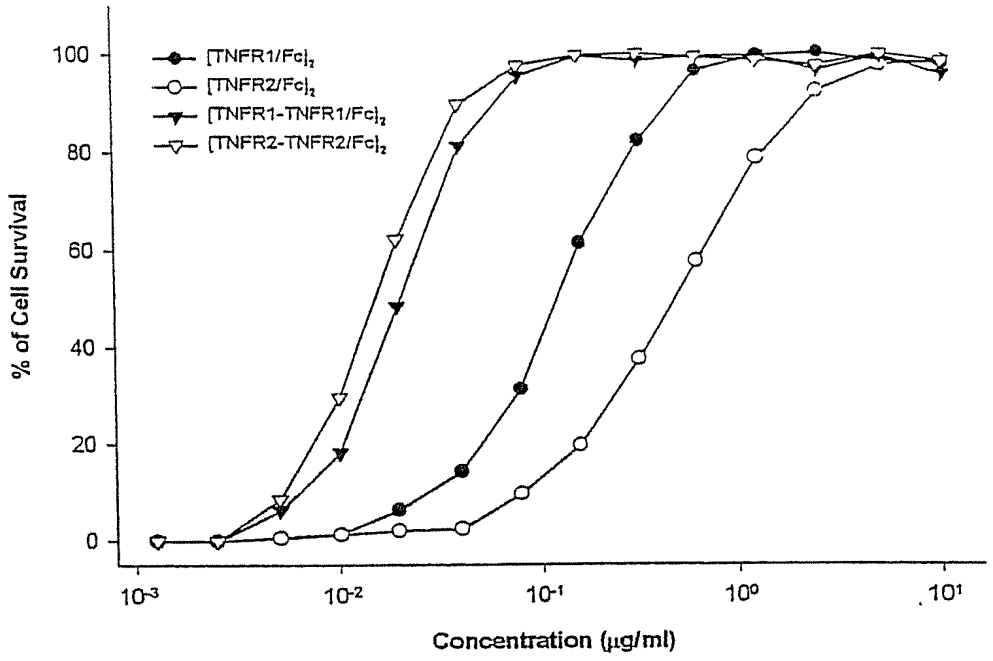

[TNFR1/Fc]2
[TNFR2/Fc]2
[TNFR1-TNFR1/Fc]2
[TNFR2-TNFR2/Fc]2
% of Cell Survival
100
80
60
40
20
0
10-3
10-2
10-1
100
101
Concentration (μg/ml)

FIG. 18

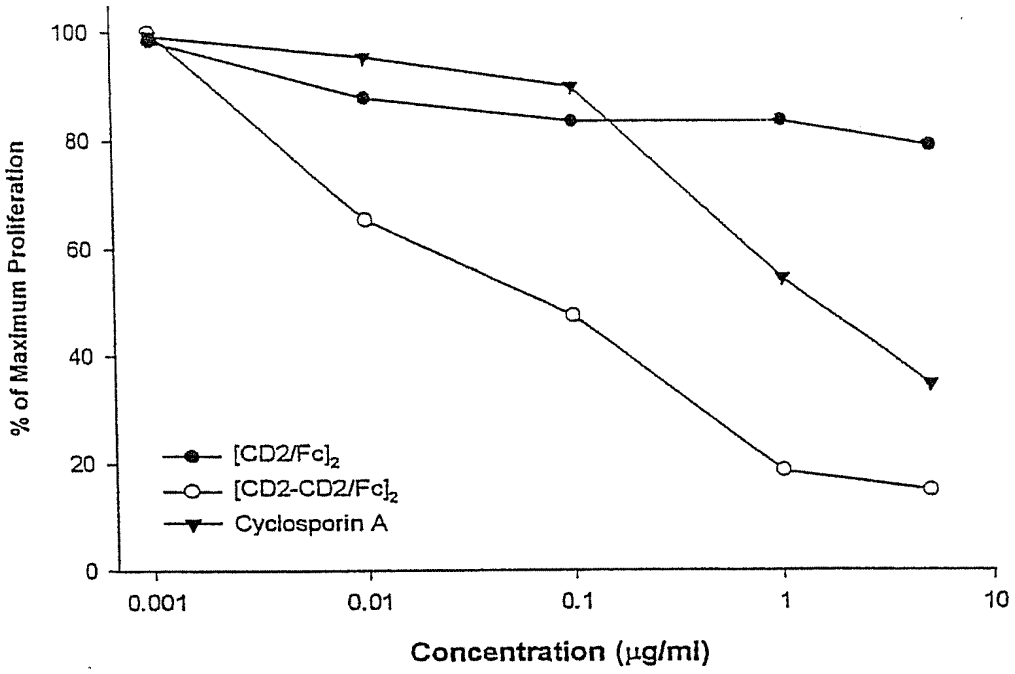

100
80
60
40
20
0
% of Maximum Proliferation
[CD2/Fc]2
[CD2-CD2/Fc]2
Cyclosporin A
0.001
0.01
0.1
1
10
Concentration (μg/ml)

FIG. 19

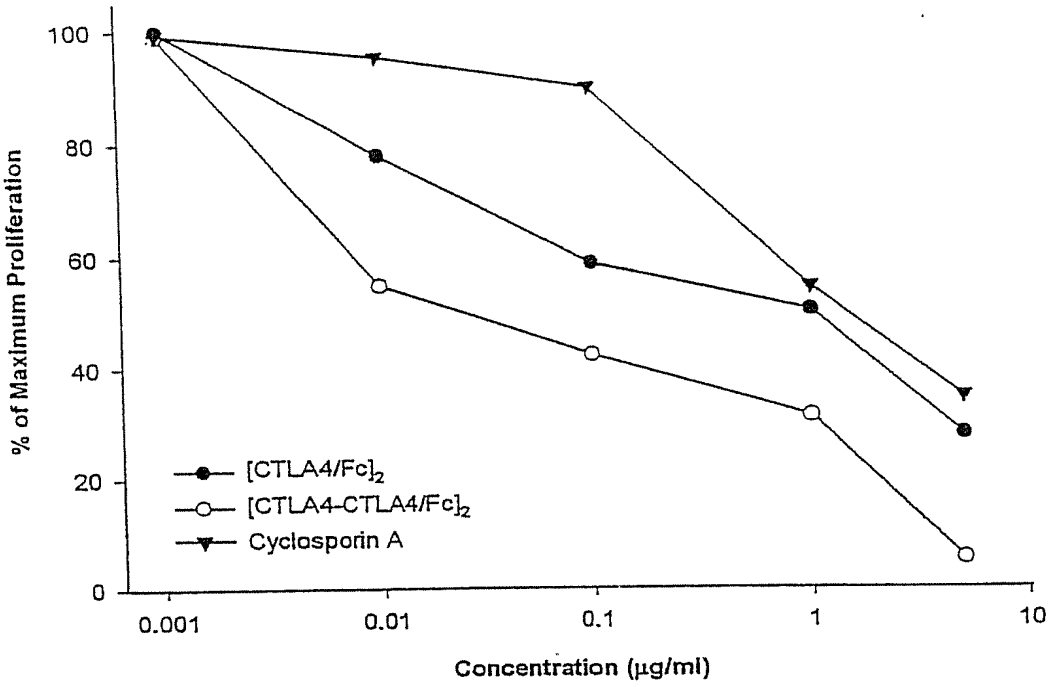

100
80
60
40
20
0
% of Maximum Proliferation
0.001
0.01
0.1
1
10
Concentration (μg/ml)
[CTLA4/Fc]2
[CTLA4-CTLA4/Fc]2
Cyclosporin A

FIG. 20

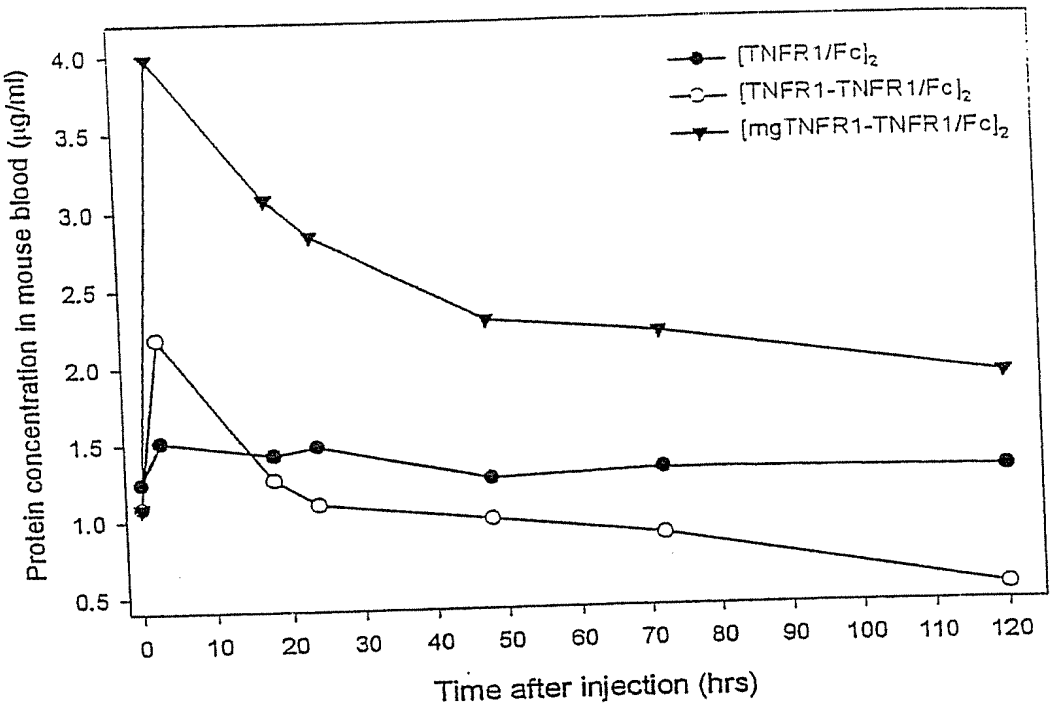

[TNFR1/Fc]2
[TNFR1-TNFR1/Fc]2
[mgTNFR1-TNFR1/Fc]2
Protein concentration in mouse blood (μg/ml)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
10
20
30
40
50
60
70
80
90
100
110
120
Time after injection (hrs)

FIG. 21

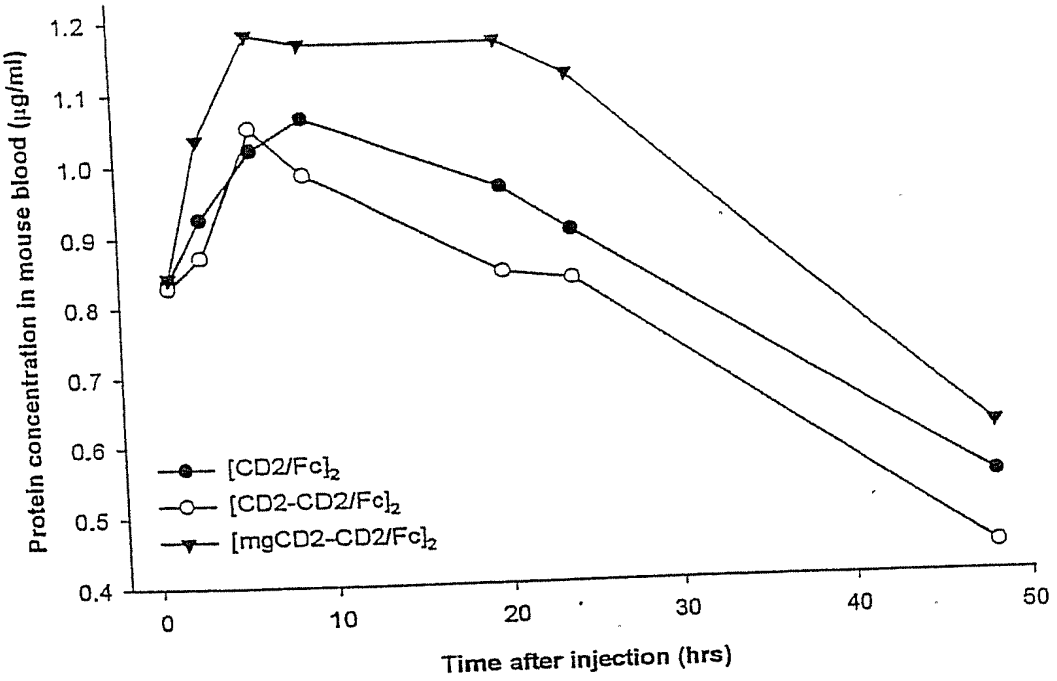

Protein concentration in mouse blood (μg/ml)
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0
10
20
30
40
50
Time after injection (hrs)
[CD2/Fc]2
[CD2-CD2/Fc]2
[mgCD2-CD2/Fc]2

FIG. 22

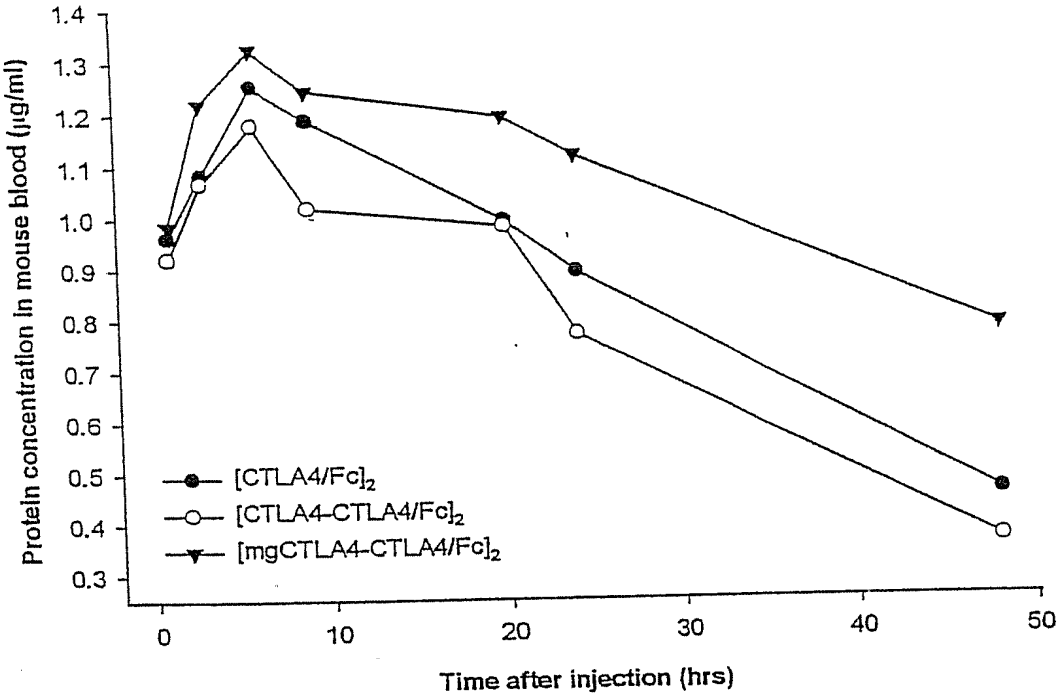
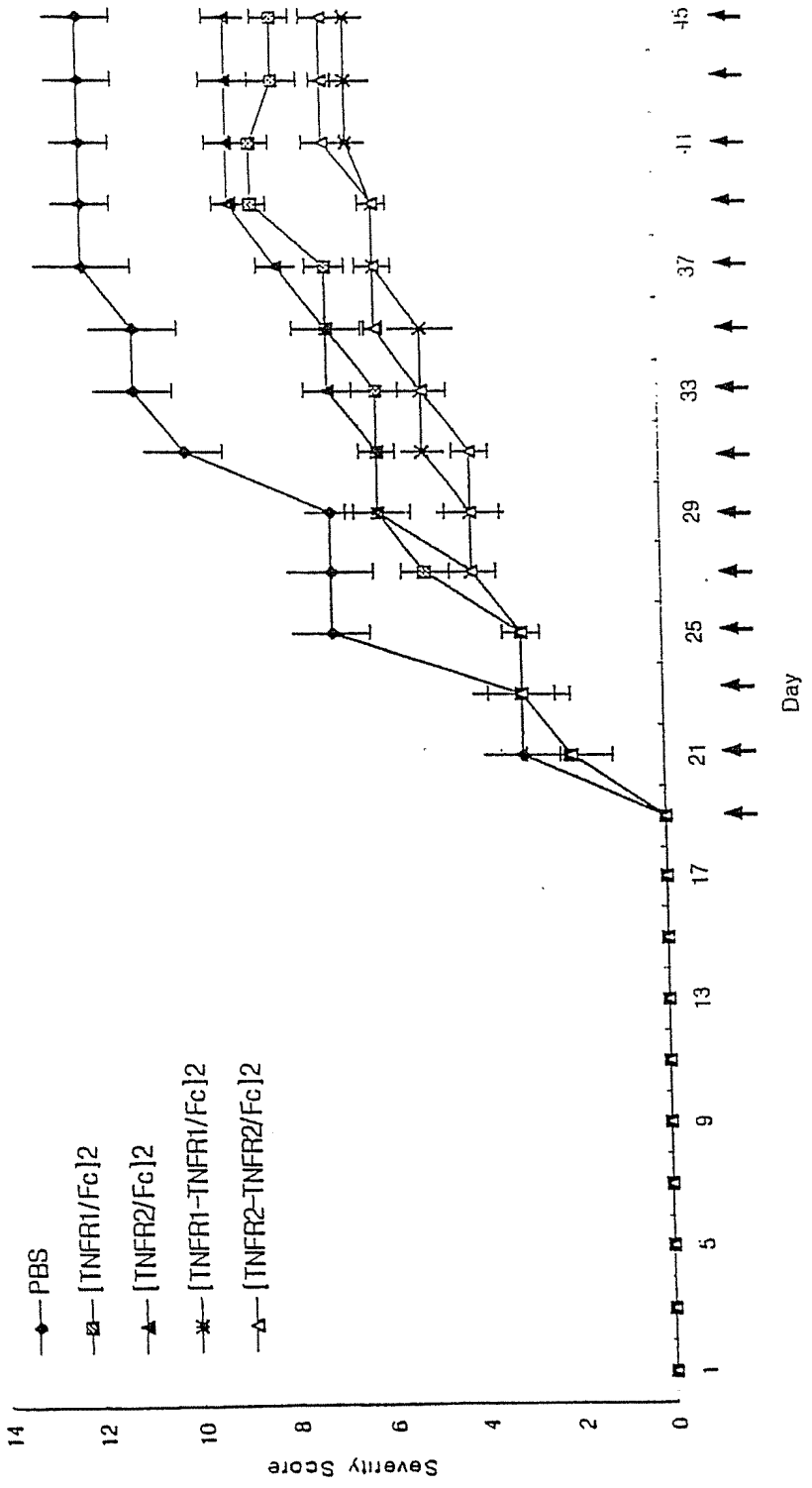

Protein concentration in mouse blood (μg/ml)
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0
10
20
30
40
50
Time after injection (hrs)
[CTLA4/Fc]2
[CTLA4-CTLA4/Fc]2
[mgCTLA4-CTLA4/Fc]2

FIG. 23


PBS
[TNFR1/Fc]2
[TNFR2/Fc]2
[TNFR1-TNFR1/Fc]2
[TNFR2-TNFR2/Fc]2
Severity Score
Day
0
2
4
6
8
10
12
14
1
5
9
13
17
21
25
29
33
37
41
45

Sequence Listing

<110> MeDexGen Inc.
CHUNG, Yong Hoon
HAN, Ji Woong
LEE, Hye Ja
CHOI, Eun Yong
KIM, Jin Mi
YIM, Soo Bin

<120> Method of manufacturing Ig-fusion proteins by concatamerization, TNFR/Fc, CD2/Fc, CTLA4/Fc fusion proteins manufactured by the method, DNA coding the proteins, vectors including the DNA, and cells transformed by the vectorTOR

<160> 52

<170> KopatentIn 1.71

<210> 1
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<213> Homo sapiens

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<223> TNFR1-IgG

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<223> Hinge, CH2, CH3 region

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<223> N-linked glycosylation site

Sequence Listing

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<223> N-linked glycosylation site

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<223> N-linked glycosylation site

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<223> PCR primer SEQ ID : 25 binding site

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<222> (616)..(652)
<223> PCR primer SEQ ID : 26(antisense) binding site

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<222> (616)..(651)
<223> PCR primer SEQ ID : 27 binding site

<220>
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<222> (1312)..(1335)
<223> PCR primer SEQ ID : 28(antisense) binding site

<220>
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<222> (1)..(60)
<223> signal peptide

Sequence Listing

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atg ggc ctc tcc acc gtg cct gac ctg ctg ctg ccg ctg gtg ctc ctg          48
Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
  1               5                  10                  15

gag ctg ttg gtg gga ata tac ccc tca ggg gtt att gga ctg gtc cct          96
Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
             20                  25                  30

cac cta ggg gac agg gag aag aga gat agt gtg tgt ccc caa gga aaa         144
His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
         35                  40                  45

tat atc cac cct caa aat aat tcg att tgc tgt acc aag tgc cac aaa         192
Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
     50                  55                  60

gga acc tac ttg tac aat gac tgt cca ggc ccg ggg cag gat acg gac         240
Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
 65                  70                  75                  80

tgc agg gag tgt gag agc ggc tcc ttc acc gct tca gaa aac cac ctc         288
Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
                 85                  90                  95

aga cac tgc ctc agc tgc tcc aaa tgc cga aag gaa atg ggt cag gtg         336
Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
            100                 105                 110

gag atc tct tct tgc aca gtg gac cgg gac acc gtg tgt ggc tgc agg         384
Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
        115                 120                 125

aag aac cag tac cgg cat tat tgg agt gaa aac ctt ttc cag tgc ttc         432
Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
    130                 135                 140

aat tgc agc ctc tgc ctc aat ggg acc gtg cac ctc tcc tgc cag gag         480
```

Sequence Listing

```
Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145                 150                 155                 160

aaa cag aac acc gtg tgc acc tgc cat gca ggt ttc ttt cta aga gaa      528
Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
                165                 170                 175

aac gag tgt gtc tcc tgt agt aac tgt aag aaa agc ctg gag tgc acg      576
Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
            180                 185                 190

aag ttg tgc cta ccc cag att gag aat gtt aag ggc act gag gac tca      624
Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
        195                 200                 205

ggc acc aca gca gag ccc aaa tct tgt gac aaa act cac aca tgc cca      672
Gly Thr Thr Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro
    210                 215                 220

ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc      720
Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
225                 230                 235                 240

ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc      768
Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
                245                 250                 255

aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc      816
Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
            260                 265                 270

aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg      864
Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
        275                 280                 285

cgg gag gag cag tac aac agc acg tac cgg gtg gtc agc gtc ctc acc      912
Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
    290                 295                 300

gtc ctg cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc      960
```

Sequence Listing

```
Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
305                 310                 315                 320

tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc        1008
Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
                325                 330                 335

aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg        1056
Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
            340                 345                 350

gat gag ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc        1104
Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
        355                 360                 365

ttc tat ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg        1152
Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
    370                 375                 380

gag aac aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc        1200
Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
385                 390                 395                 400

tcc ttc ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag        1248
Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
                405                 410                 415

ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac        1296
Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
            420                 425                 430

tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa          tga            1335
Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
        435                 440
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<210> 2
<211> 444
<212> PRT
<213> Homo sapiens

Sequence Listing

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Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
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Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
20 25 30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
35 40 45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
50 55 60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65 70 75 80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
85 90 95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
100 105 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
115 120 125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
130 135 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145 150 155 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
165 170 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
180 185 190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Ser
195 200 205

Sequence Listing

Gly Thr Thr Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro
210 215 220

Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe
225 230 235 240

Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val
245 250 255

Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe
260 265 270

Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro
275 280 285

Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr
290 295 300

Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val
305 310 315 320

Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala
325 330 335

Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg
340 345 350

Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly
355 360 365

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro
370 375 380

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser
385 390 395 400

Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln
405 410 415

Sequence Listing

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His
420 425 430

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
435 440

<210> 3
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<223> Hinge, CH2, CH3 region

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Sequence Listing

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<223> PCR primer SEQ ID : 31 binding site

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atg gcg ccc gtc gcc gtc tgg gcc gcg ctg gcc gtc gga ctg gag ctc          48
Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu
  1               5                  10                  15

tgg gct gcg gcg cac gcc ttg ccc gcc cag gtg gca ttt aca ccc tac          96
Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
             20                  25                  30

gcc ccg gag ccc ggg agc aca tgc cgg ctc aga gaa tac tat gac cag         144
Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
         35                  40                  45

aca gct cag atg tgc tgc agc aaa tgc tcg ccg ggc caa cat gca aaa         192
```

Sequence Listing

```
Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
     50                  55                  60

gtc ttc tgt acc aag acc tcg gac acc gtg tgt gac tcc tgt gag gac        240
Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
 65                  70                  75                  80

agc aca tac acc cag ctc tgg aac tgg gtt ccc gag tgc ttg agc tgt        288
Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
                85                  90                  95

ggc tcc cgc tgt agc tct gac cag gtg gaa act caa gcc tgc act cgg        336
Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
            100                 105                 110

gaa cag aac cgc atc tgc acc tgc agg ccc ggc tgg tac tgc gcg ctg        384
Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu
        115                 120                 125

agc aag cag gag ggg tgc cgg ctg tgc gcg ccg ctg cgc aag tgc cgc        432
Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
    130                 135                 140

ccg ggc ttc ggc gtg gcc aga cca gga act gaa aca tca gac gtg gtg        480
Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val
145                 150                 155                 160

tgc aag ccc tgt gcc ccg ggg acg ttc tcc aac acg act tca tcc acg        528
Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr
                165                 170                 175

gat att tgc agg ccc cac cag atc tgt aac gtg gtg gcc atc cct ggg        576
Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly
            180                 185                 190

aat gca agc atg gat gca gtc tgc acg tcc acg tcc ccc acc cgg agt        624
Asn Ala Ser Met Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Arg Ser
        195                 200                 205

atg gcc cca ggg gca gta cac tta ccc cag cca gtg tcc aca cga tcc        672
```

Sequence Listing

```
Met Ala Pro Gly Ala Val His Leu Pro Gln Pro Val Ser Thr Arg Ser
    210                 215                 220

caa cac acg cag cca act cca gaa ccc agc act gct cca agc acc tcc        720
Gln His Thr Gln Pro Thr Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser
225                 230                 235                 240

ttc ctg ctc cca atg ggc ccc agc ccc cca gct gaa ggg agc act ggc        768
Phe Leu Leu Pro Met Gly Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly
                245                 250                 255

gac gca gag ccc aaa tct tgt gac aaa act cac aca tgc cca ccg tgc        816
Asp Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            260                 265                 270

cca gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca        864
Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        275                 280                 285

aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc        912
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    290                 295                 300

gtg gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg        960
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
305                 310                 315                 320

tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag       1008
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                325                 330                 335

gag cag tac aac agc acg tac cgg gtg gtc agc gtc ctc acc gtc ctg       1056
Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            340                 345                 350

cac cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac       1104
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        355                 360                 365

aaa gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg       1152
```

Sequence Listing

```
Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    370                 375                 380

cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag      1200
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
385                 390                 395                 400

ctg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat      1248
Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                405                 410                 415

ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac      1296
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            420                 425                 430

aac tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc tcc ttc      1344
Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Ser Phe
        435                 440                 445

ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac      1392
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
    450                 455                 460

gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg      1440
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
465                 470                 475                 480

cag aag agc ctc tcc ctg tct ccg ggt aaa     tga                     1473
Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
                485                 490
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<210> 4
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<212> PRT
<213> Homo sapiens

<400> 4

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Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu
  1               5                  10                  15
```

Sequence Listing

```
Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
            20                  25                  30

Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
        35                  40                  45

Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
    50                  55                  60

Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
65                  70                  75                  80

Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
                85                  90                  95

Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
            100                 105                 110

Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu
        115                 120                 125

Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
    130                 135                 140

Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val
145                 150                 155                 160

Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr
                165                 170                 175

Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly
            180                 185                 190

Asn Ala Ser Met Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Arg Ser
        195                 200                 205

Met Ala Pro Gly Ala Val His Leu Pro Gln Pro Val Ser Thr Arg Ser
    210                 215                 220
```

Sequence Listing

```
Gln His Thr Gln Pro Thr Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser
225                 230                 235                 240

Phe Leu Leu Pro Met Gly Pro Ser Pro Pro Ala Glu Gly Ser Thr Gly
                245                 250                 255

Asp Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
            260                 265                 270

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
        275                 280                 285

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
    290                 295                 300

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
305                 310                 315                 320

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
                325                 330                 335

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
            340                 345                 350

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
        355                 360                 365

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
    370                 375                 380

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
385                 390                 395                 400

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
                405                 410                 415

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
            420                 425                 430

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Ser Phe
```

Sequence Listing

435 440 445

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
450 455 460

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
465 470 475 480

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
485 490

<210> 5
<211> 1887
<212> DNA
<213> Homo sapiens

<220>
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<222> (1)..(1884)
<223> TNFR1-TNFR1-IgG

<220>
<221> C_region
<222> (1716)..(1887)
<223> Hinge, CH2, CH3 region

<220>
<221> misc_signal
<222> (160)..(168)
<223> N-linked glycosylation site

<220>
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<222> (433)..(441)
<223> N-linked glycosylation site

Sequence Listing

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<222> (451)..(459)
<223> N-linked glycosylation site

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<222> (631)..(639)
<223> N-linked glycosylation site

<220>
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<222> (712)..(720)
<223> N-linked glycosylation site

<220>
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<222> (985)..(993)
<223> N-linked glycosylation site

<220>
<221> misc_signal
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<223> N-linked glycosylation site

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<223> PCR primer SEQ ID : 25 binding site

<220>
<221> primer_bind
<222> (592)..(628)

Sequence Listing

<223> PCR primer SEQ ID : 33(antisense) binding site

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<223> PCR primer SEQ ID : 26(antisense) binding site

<220>
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<222> (1168)..(1204)
<223> PCR primer SEQ ID : 27 binding site

<220>
<221> primer_bind
<222> (1864)..(1887)
<223> PCR primer SEQ ID : 28(antisense) binding site

<220>
<221> sig_peptide
<222> (1)..(60)
<223> signal peptide

<400> 5

```
atg ggc ctc tcc acc gtg cct gac ctg ctg ctg ccg ctg gtg ctc ctg          48
Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
  1               5                  10                  15

gag ctg ttg gtg gga ata tac ccc tca ggg gtt att gga ctg gtc cct          96
Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
```

Sequence Listing

```
                20                  25                  30

cac cta ggg gac agg gag aag aga gat agt gtg tgt ccc caa gga aaa      144
His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
        35                  40                  45

tat atc cac cct caa aat aat tcg att tgc tgt acc aag tgc cac aaa      192
Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
    50                  55                  60

gga acc tac ttg tac aat gac tgt cca ggc ccg ggg cag gat acg gac      240
Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65                  70                  75                  80

tgc agg gag tgt gag agc ggc tcc ttc acc gct tca gaa aac cac ctc      288
Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
                85                  90                  95

aga cac tgc ctc agc tgc tcc aaa tgc cga aag gaa atg ggt cag gtg      336
Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
            100                 105                 110

gag atc tct tct tgc aca gtg gac cgg gac acc gtg tgt ggc tgc agg      384
Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
        115                 120                 125

aag aac cag tac cgg cat tat tgg agt gaa aac ctt ttc cag tgc ttc      432
Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
    130                 135                 140

aat tgc agc ctc tgc ctc aat ggg acc gtg cac ctc tcc tgc cag gag      480
Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145                 150                 155                 160

aaa cag aac acc gtg tgc acc tgc cat gca ggt ttc ttt cta aga gaa      528
Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
                165                 170                 175

aac gag tgt gtc tcc tgt agt aac tgt aag aaa agc ctg gag tgc acg      576
Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
```

Sequence Listing

```
                180                 185                 190

aag ttg tgc cta ccc cag att gag aat gtt aag ggc act gag gac gga          624
Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Gly
        195                 200                 205

tcc ggg aac att tca ctg gtc cct cac cta ggg gac agg gag aag aga          672
Ser Gly Asn Ile Ser Leu Val Pro His Leu Gly Asp Arg Glu Lys Arg
    210                 215                 220

gat agt gtg tgt ccc caa gga aaa tat atc cac cct caa aat aat tcg          720
Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser
225                 230                 235                 240

att tgc tgt acc aag tgc cac aaa gga acc tac ttg tac aat gac tgt          768
Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys
                245                 250                 255

cca ggc ccg ggg cag gat acg gac tgc agg gag tgt gag agc ggc tcc          816
Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser
            260                 265                 270

ttc acc gct tca gaa aac cac ctc aga cac tgc ctc agc tgc tcc aaa          864
Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys
        275                 280                 285

tgc cga aag gaa atg ggt cag gtg gag atc tct tct tgc aca gtg gac          912
Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp
    290                 295                 300

cgg gac acc gtg tgt ggc tgc agg aag aac cag tac cgg cat tat tgg          960
Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp
305                 310                 315                 320

agt gaa aac ctt ttc cag tgc ttc aat tgc agc ctc tgc ctc aat ggg         1008
Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly
                325                 330                 335

acc gtg cac ctc tcc tgc cag gag aaa cag aac acc gtg tgc acc tgc         1056
Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys
```

Sequence Listing

```
            340                 345                 350

cat gca ggt ttc ttt cta aga gaa aac gag tgt gtc tcc tgt agt aac        1104
His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn
        355                 360                 365

tgt aag aaa agc ctg gag tgc acg aag ttg tgc cta ccc cag att gag        1152
Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu
    370                 375                 380

aat gtt aag ggc act gag gac tca ggc acc aca gca gag ccc aaa tct        1200
Asn Val Lys Gly Thr Glu Asp Ser Gly Thr Thr Ala Glu Pro Lys Ser
385                 390                 395                 400

tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg        1248
Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
                405                 410                 415

ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc        1296
Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
            420                 425                 430

atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc        1344
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
        435                 440                 445

cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag        1392
His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
    450                 455                 460

gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg        1440
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
465                 470                 475                 480

tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat        1488
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
                485                 490                 495

ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc        1536
Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
```

Sequence Listing

```
            500                 505                 510

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag        1584
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
        515                 520                 525

gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc        1632
Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
    530                 535                 540

agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg        1680
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
545                 550                 555                 560

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct        1728
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
                565                 570                 575

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc        1776
Pro Val Leu Asp Ser Asp Gly Ser Ser Phe Leu Tyr Ser Lys Leu Thr
            580                 585                 590

gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg        1824
Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
        595                 600                 605

atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg        1872
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
    610                 615                 620

tct ccg ggt aaa           tga                                          1887
Ser Pro Gly Lys
625
```

<210> 6
<211> 628
<212> PRT
<213> Homo sapiens

Sequence Listing

<400> 6

```
Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
 1               5                  10                  15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
            20                  25                  30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
        35                  40                  45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
    50                  55                  60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65                  70                  75                  80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
                85                  90                  95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
            100                 105                 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
        115                 120                 125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
    130                 135                 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145                 150                 155                 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
                165                 170                 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Leu Glu Cys Thr
            180                 185                 190

Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly Thr Glu Asp Gly
        195                 200                 205
```

Sequence Listing

Ser Gly Asn Ile Ser Leu Val Pro His Leu Gly Asp Arg Glu Lys Arg
210 215 220

Asp Ser Val Cys Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser
225 230 235 240

Ile Cys Cys Thr Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys
245 250 255

Pro Gly Pro Gly Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser
260 265 270

Phe Thr Ala Ser Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys
275 280 285

Cys Arg Lys Glu Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp
290 295 300

Arg Asp Thr Val Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp
305 310 315 320

Ser Glu Asn Leu Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly
325 330 335

Thr Val His Leu Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys
340 345 350

His Ala Gly Phe Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn
355 360 365

Cys Lys Lys Ser Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu
370 375 380

Asn Val Lys Gly Thr Glu Asp Ser Gly Thr Thr Ala Glu Pro Lys Ser
385 390 395 400

Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
405 410 415

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu

Sequence Listing

420 425 430

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
435 440 445

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
450 455 460

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
465 470 475 480

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
485 490 495

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
500 505 510

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
515 520 525

Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val
530 535 540

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
545 550 555 560

Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
565 570 575

Pro Val Leu Asp Ser Asp Gly Ser Ser Phe Leu Tyr Ser Lys Leu Thr
580 585 590

Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
595 600 605

Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
610 615 620

Ser Pro Gly Lys
625

Sequence Listing

<210> 7
<211> 2163
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(2160)
<223> TNFR2-TNFR2-IgG

<220>
<221> C_region
<222> (1462)..(2163)
<223> Hinge, CH2, CH3 region

<220>
<221> misc_signal
<222> (511)..(519)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (577)..(585)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (769)..(777)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (1201)..(1209)

Sequence Listing

<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (1267)..(1275)
<223> N-linked glycosylation site

<220>
<221> primer_bind
<222> (1)..(15)
<223> PCR primer SEQ ID : 29 binding site

<220>
<221> primer_bind
<222> (761)..(795)
<223> PCR primer SEQ ID : 35(antisense) binding site

<220>
<221> primer_bind
<222> (741)..(768)
<223> PCR primer SEQ ID : 34 binding site

<220>
<221> primer_bind
<222> (1444)..(1480)
<223> PCR primer SEQ ID : 30(antisense) binding site

<220>
<221> primer_bind
<222> (1444)..(1480)
<223> PCR primer SEQ ID : 31 binding site

<220>

Sequence Listing

<221> primer_bind
<222> (2141)..(2163)
<223> PCR primer SEQ ID : 28(antisense) binding site

<220>
<221> sig_peptide
<222> (1)..(66)
<223> signal peptide

<400> 7

```
atg gcg ccc gtc gcc gtc tgg gcc gcg ctg gcc gtc gga ctg gag ctc          48
Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu
  1               5                  10                  15

tgg gct gcg gcg cac gcc ttg ccc gcc cag gtg gca ttt aca ccc tac          96
Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
             20                  25                  30

gcc ccg gag ccc ggg agc aca tgc cgg ctc aga gaa tac tat gac cag         144
Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
         35                  40                  45

aca gct cag atg tgc tgc agc aaa tgc tcg ccg ggc caa cat gca aaa         192
Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
     50                  55                  60

gtc ttc tgt acc aag acc tcg gac acc gtg tgt gac tcc tgt gag gac         240
Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
 65                  70                  75                  80

agc aca tac acc cag ctc tgg aac tgg gtt ccc gag tgc ttg agc tgt         288
Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
                 85                  90                  95

ggc tcc cgc tgt agc tct gac cag gtg gaa act caa gcc tgc act cgg         336
Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
            100                 105                 110
```

Sequence Listing

```
gaa cag aac cgc atc tgc acc tgc agg ccc ggc tgg tac tgc gcg ctg      384
Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu
        115                 120                 125

agc aag cag gag ggg tgc cgg ctg tgc gcg ccg ctg cgc aag tgc cgc      432
Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
    130                 135                 140

ccg ggc ttc ggc gtg gcc aga cca gga act gaa aca tca gac gtg gtg      480
Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val
145                 150                 155                 160

tgc aag ccc tgt gcc ccg ggg acg ttc tcc aac acg act tca tcc acg      528
Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr
                165                 170                 175

gat att tgc agg ccc cac cag atc tgt aac gtg gtg gcc atc cct ggg      576
Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly
            180                 185                 190

aat gca agc atg gat gca gtc tgc acg tcc acg tcc ccc acc cgg agt      624
Asn Ala Ser Met Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Arg Ser
        195                 200                 205

atg gcc cca ggg gca gta cac tta ccc cag cca gtg tcc aca cga tcc      672
Met Ala Pro Gly Ala Val His Leu Pro Gln Pro Val Ser Thr Arg Ser
    210                 215                 220

caa cac acg cag cca act cca gaa ccc agc act gct cca agc acc tcc      720
Gln His Thr Gln Pro Thr Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser
225                 230                 235                 240

ttc ctg ctc cca atg ggc ccc agc ccc cca gct gaa ggg agc gga tcc      768
Phe Leu Leu Pro Met Gly Pro Ser Pro Pro Ala Glu Gly Ser Gly Ser
                245                 250                 255

aac gca act aca ccc tac gcc ccg gag ccc ggg agc aca tgc cgg ctc      816
Asn Ala Thr Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu
            260                 265                 270
```

Sequence Listing

```
aga gaa tac tat gac cag aca gct cag atg tgc tgc agc aaa tgc tcg          864
Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys Ser Lys Cys Ser
        275                 280                 285

ccg ggc caa cat gca aaa gtc ttc tgt acc aag acc tcg gac acc gtg          912
Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr Ser Asp Thr Val
    290                 295                 300

tgt gac tcc tgt gag gac agc aca tac acc cag ctc tgg aac tgg gtt          960
Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val
305                 310                 315                 320

ccc gag tgc ttg agc tgt ggc tcc cgc tgt agc tct gac cag gtg gaa         1008
Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser Asp Gln Val Glu
                325                 330                 335

act caa gcc tgc act cgg gaa cag aac cgc atc tgc acc tgc agg ccc         1056
Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro
            340                 345                 350

ggc tgg tac tgc gcg ctg agc aag cag gag ggg tgc cgg ctg tgc gcg         1104
Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala
        355                 360                 365

ccg ctg cgc aag tgc cgc ccg ggc ttc ggc gtg gcc aga cca gga act         1152
Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala Arg Pro Gly Thr
    370                 375                 380

gaa aca tca gac gtg gtg tgc aag ccc tgt gcc ccg ggg acg ttc tcc         1200
Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser
385                 390                 395                 400

aac acg act tca tcc acg gat att tgc agg ccc cac cag atc tgt aac         1248
Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His Gln Ile Cys Asn
                405                 410                 415

gtg gtg gcc atc cct ggg aat gca agc atg gat gca gtc tgc acg tcc         1296
Val Val Ala Ile Pro Gly Asn Ala Ser Met Asp Ala Val Cys Thr Ser
            420                 425                 430
```

Sequence Listing

```
acg tcc ccc acc cgg agt atg gcc cca ggg gca gta cac tta ccc cag        1344
Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val His Leu Pro Gln
        435                 440                 445

cca gtg tcc aca cga tcc caa cac acg cag cca act cca gaa ccc agc        1392
Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr Pro Glu Pro Ser
    450                 455                 460

act gct cca agc acc tcc ttc ctg ctc cca atg ggc ccc agc ccc cca        1440
Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly Pro Ser Pro Pro
465                 470                 475                 480

gct gaa ggg agc act ggc gac gca gag ccc aaa tct tgt gac aaa act        1488
Ala Glu Gly Ser Thr Gly Asp Ala Glu Pro Lys Ser Cys Asp Lys Thr
                485                 490                 495

cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca        1536
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
            500                 505                 510

gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg        1584
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
        515                 520                 525

acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct        1632
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
    530                 535                 540

gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc        1680
Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
545                 550                 555                 560

aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgg gtg gtc        1728
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
                565                 570                 575

agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac        1776
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
            580                 585                 590
```

Sequence Listing

```
aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc        1824
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
        595                 600                 605

atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg        1872
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
    610                 615                 620

ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc        1920
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
625                 630                 635                 640

ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc        1968
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
                645                 650                 655

aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac        2016
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
            660                 665                 670

tcc gac ggc tcc tcc ttc ctc tac agc aag ctc acc gtg gac aag agc        2064
Ser Asp Gly Ser Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
        675                 680                 685

agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct        2112
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
    690                 695                 700

ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa        2160
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
705                 710                 715                 720

tga                                                                    2163
```

<210> 8
<211> 720
<212> PRT
<213> Homo sapiens

Sequence Listing

<400> 8

```
Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu
 1               5                  10                  15

Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
            20                  25                  30

Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
        35                  40                  45

Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
    50                  55                  60

Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
65                  70                  75                  80

Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
                85                  90                  95

Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
            100                 105                 110

Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu
        115                 120                 125

Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
    130                 135                 140

Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val
145                 150                 155                 160

Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr
                165                 170                 175

Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly
            180                 185                 190

Asn Ala Ser Met Asp Ala Val Cys Thr Ser Thr Ser Pro Thr Arg Ser
        195                 200                 205
```

Sequence Listing

Met Ala Pro Gly Ala Val His Leu Pro Gln Pro Val Ser Thr Arg Ser
210 215 220

Gln His Thr Gln Pro Thr Pro Glu Pro Ser Thr Ala Pro Ser Thr Ser
225 230 235 240

Phe Leu Leu Pro Met Gly Pro Ser Pro Pro Ala Glu Gly Ser Gly Ser
245 250 255

Asn Ala Thr Thr Pro Tyr Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu
260 265 270

Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys Ser Lys Cys Ser
275 280 285

Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr Ser Asp Thr Val
290 295 300

Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val
305 310 315 320

Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser Asp Gln Val Glu
325 330 335

Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro
340 345 350

Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala
355 360 365

Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala Arg Pro Gly Thr
370 375 380

Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser
385 390 395 400

Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His Gln Ile Cys Asn
405 410 415

Val Val Ala Ile Pro Gly Asn Ala Ser Met Asp Ala Val Cys Thr Ser

Sequence Listing

420 425 430

Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val His Leu Pro Gln
435 440 445

Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr Pro Glu Pro Ser
450 455 460

Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly Pro Ser Pro Pro
465 470 475 480

Ala Glu Gly Ser Thr Gly Asp Ala Glu Pro Lys Ser Cys Asp Lys Thr
485 490 495

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
500 505 510

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
515 520 525

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
530 535 540

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
545 550 555 560

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
565 570 575

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
580 585 590

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
595 600 605

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
610 615 620

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
625 630 635 640

Sequence Listing

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
645 650 655

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
660 665 670

Ser Asp Gly Ser Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
675 680 685

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
690 695 700

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
705 710 715 720

<210> 9
<211> 1827
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1824)
<223> mgTNFR1-TNFR1-IgG

<220>
<221> C_region
<222> (1126)..(1827)
<223> Hinge, CH2, CH3 region

<220>
<221> misc_signal
<222> (160)..(168)
<223> N-linked glycosylation site

Sequence Listing

<220>
<221> misc_signal
<222> (433)..(441)
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<222> (451)..(459)
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<220>
<221> misc_signal
<222> (565)..(573)
<223> N-linked glycosylation site

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<222> (574)..(582)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (592)..(600)
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<220>
<221> misc_signal
<222> (610)..(618)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (925)..(933)
<223> N-linked glycosylation site

Sequence Listing

<220>
<221> misc_signal
<222> (943)..(951)
<223> N-linked glycosylation site

<220>
<221> primer_bind
<222> (1)..(15)
<223> PCR primer SEQ ID : 25 binding site

<220>
<221> primer_bind
<222> (545)..(606)
<223> PCR primer SEQ ID : 37(antisense) binding site

<220>
<221> primer_bind
<222> (559)..(621)
<223> PCR primer SEQ ID : 36 binding site

<220>
<221> primer_bind
<222> (1108)..(1144)
<223> PCR primer SEQ ID : 26(antisense) binding site

<220>
<221> primer_bind
<222> (1108)..(1144)
<223> PCR primer SEQ ID : 27 binding site

<220>
<221> primer_bind

Sequence Listing

<222> (1804)..(1827)
<223> PCR primer SEQ ID : 28(antisense) binding site

<220>
<221> sig_peptide
<222> (1)..(60)
<223> signal peptide

<400> 9

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atg ggc ctc tcc acc gtg cct gac ctg ctg ctg ccg ctg gtg ctc ctg          48
Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
  1               5                  10                  15

gag ctg ttg gtg gga ata tac ccc tca ggg gtt att gga ctg gtc cct          96
Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
             20                  25                  30

cac cta ggg gac agg gag aag aga gat agt gtg tgt ccc caa gga aaa         144
His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
         35                  40                  45

tat atc cac cct caa aat aat tcg att tgc tgt acc aag tgc cac aaa         192
Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
     50                  55                  60

gga acc tac ttg tac aat gac tgt cca ggc ccg ggg cag gat acg gac         240
Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
 65                  70                  75                  80

tgc agg gag tgt gag agc ggc tcc ttc acc gct tca gaa aac cac ctc         288
Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
                 85                  90                  95

aga cac tgc ctc agc tgc tcc aaa tgc cga aag gaa atg ggt cag gtg         336
Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
            100                 105                 110

gag atc tct tct tgc aca gtg gac cgg gac acc gtg tgt ggc tgc agg         384
```

Sequence Listing

```
Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
        115                 120                 125

aag aac cag tac cgg cat tat tgg agt gaa aac ctt ttc cag tgc ttc      432
Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
    130                 135                 140

aat tgc agc ctc tgc ctc aat ggg acc gtg cac ctc tcc tgc cag gag      480
Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu
145                 150                 155                 160

aaa cag aac acc gtg tgc acc tgc cat gca ggt ttc ttt cta aga gaa      528
Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
                165                 170                 175

aac gag tgt gtc tcc tgt agt aac tgt aag aaa agc aac gag acc aac      576
Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Asn Glu Thr Asn
            180                 185                 190

aag acc tgc cta cac aac ggg tcc agg gag aag aac gat agt gtg tgt      624
Lys Thr Cys Leu His Asn Gly Ser Arg Glu Lys Asn Asp Ser Val Cys
        195                 200                 205

ccc caa gga aaa tat atc cac cct caa aat aat tcg att tgc tgt acc      672
Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr
    210                 215                 220

aag tgc cac aaa gga acc tac ttg tac aat gac tgt cca ggc ccg ggg      720
Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly
225                 230                 235                 240

cag gat acg gac tgc agg gag tgt gag agc ggc tcc ttc acc gct tca      768
Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser
                245                 250                 255

gaa aac cac ctc aga cac tgc ctc agc tgc tcc aaa tgc cga aag gaa      816
Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu
            260                 265                 270

atg ggt cag gtg gag atc tct tct tgc aca gtg gac cgg gac acc gtg      864
```

Sequence Listing

```
Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val
        275                 280                 285

tgt ggc tgc agg aag aac cag tac cgg cat tat tgg agt gaa aac ctt      912
Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu
    290                 295                 300

ttc cag tgc ttc aat tgc agc ctc tgc ctc aat ggg acc gtg cac ctc      960
Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu
305                 310                 315                 320

tcc tgc cag gag aaa cag aac acc gtg tgc acc tgc cat gca ggt ttc     1008
Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe
                325                 330                 335

ttt cta aga gaa aac gag tgt gtc tcc tgt agt aac tgt aag aaa agc     1056
Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser
            340                 345                 350

ctg gag tgc acg aag ttg tgc cta ccc cag att gag aat gtt aag ggc     1104
Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly
        355                 360                 365

act gag gac tca ggc acc aca gca gag ccc aaa tct tgt gac aaa act     1152
Thr Glu Asp Ser Gly Thr Thr Ala Glu Pro Lys Ser Cys Asp Lys Thr
    370                 375                 380

cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg gga ccg tca     1200
His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
385                 390                 395                 400

gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg atc tcc cgg     1248
Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
                405                 410                 415

acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac gaa gac cct     1296
Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
            420                 425                 430

gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg cat aat gcc     1344
```

Sequence Listing

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
435 440 445

aag aca aag ccg cgg gag gag cag tac aac agc acg tac cgg gtg gtc 1392
Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
450 455 460

agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc aag gag tac 1440
Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
465 470 475 480

aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc gag aaa acc 1488
Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
485 490 495

atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg tac acc ctg 1536
Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
500 505 510

ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc ctg acc tgc 1584
Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
515 520 525

ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc 1632
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
530 535 540

aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac 1680
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
545 550 555 560

tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg gac aag agc 1728
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
565 570 575

agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct 1776
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
580 585 590

ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct ccg ggt aaa 1824

Sequence Listing

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
595 600 605

tga 1827

<210> 10
<211> 608
<212> PRT
<213> Homo sapiens

<400> 10
Met Gly Leu Ser Thr Val Pro Asp Leu Leu Leu Pro Leu Val Leu Leu
1 5 10 15

Glu Leu Leu Val Gly Ile Tyr Pro Ser Gly Val Ile Gly Leu Val Pro
20 25 30

His Leu Gly Asp Arg Glu Lys Arg Asp Ser Val Cys Pro Gln Gly Lys
35 40 45

Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr Lys Cys His Lys
50 55 60

Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly Gln Asp Thr Asp
65 70 75 80

Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser Glu Asn His Leu
85 90 95

Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu Met Gly Gln Val
100 105 110

Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val Cys Gly Cys Arg
115 120 125

Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu Phe Gln Cys Phe
130 135 140

Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu Ser Cys Gln Glu

Sequence Listing

145 150 155 160

Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe Phe Leu Arg Glu
165 170 175

Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser Asn Glu Thr Asn
180 185 190

Lys Thr Cys Leu His Asn Gly Ser Arg Glu Lys Asn Asp Ser Val Cys
195 200 205

Pro Gln Gly Lys Tyr Ile His Pro Gln Asn Asn Ser Ile Cys Cys Thr
210 215 220

Lys Cys His Lys Gly Thr Tyr Leu Tyr Asn Asp Cys Pro Gly Pro Gly
225 230 235 240

Gln Asp Thr Asp Cys Arg Glu Cys Glu Ser Gly Ser Phe Thr Ala Ser
245 250 255

Glu Asn His Leu Arg His Cys Leu Ser Cys Ser Lys Cys Arg Lys Glu
260 265 270

Met Gly Gln Val Glu Ile Ser Ser Cys Thr Val Asp Arg Asp Thr Val
275 280 285

Cys Gly Cys Arg Lys Asn Gln Tyr Arg His Tyr Trp Ser Glu Asn Leu
290 295 300

Phe Gln Cys Phe Asn Cys Ser Leu Cys Leu Asn Gly Thr Val His Leu
305 310 315 320

Ser Cys Gln Glu Lys Gln Asn Thr Val Cys Thr Cys His Ala Gly Phe
325 330 335

Phe Leu Arg Glu Asn Glu Cys Val Ser Cys Ser Asn Cys Lys Lys Ser
340 345 350

Leu Glu Cys Thr Lys Leu Cys Leu Pro Gln Ile Glu Asn Val Lys Gly
355 360 365

Sequence Listing

Thr Glu Asp Ser Gly Thr Thr Ala Glu Pro Lys Ser Cys Asp Lys Thr
370 375 380

His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
385 390 395 400

Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
405 410 415

Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
420 425 430

Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
435 440 445

Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
450 455 460

Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
465 470 475 480

Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
485 490 495

Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
500 505 510

Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys
515 520 525

Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
530 535 540

Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp
545 550 555 560

Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
565 570 575

Sequence Listing

Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala
580 585 590

Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
595 600 605

<210> 11
<211> 1980
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1977)
<223> mgTNFR2-TNFR2-IgG

<220>
<221> C_region
<222> (1279)..(1980)
<223> Hinge, CH2, CH3 region

<220>
<221> misc_signal
<222> (511)..(519)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (577)..(585)
<223> N-linked glycosylation site

<220>
<221> misc_signal

Sequence Listing

<222> (595)..(603)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (616)..(624)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (1018)..(1026)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (1084)..(1092)
<223> N-linked glycosylation site

<220>
<221> primer_bind
<222> (1)..(15)
<223> PCR primer SEQ ID : 29 binding site

<220>
<221> primer_bind
<222> (586)..(627)
<223> PCR primer SEQ ID : 39(antisense) binding site

<220>
<221> primer_bind
<222> (586)..(630)
<223> PCR primer SEQ ID : 38 binding site

Sequence Listing

<220>
<221> primer_bind
<222> (1261)..(1296)
<223> PCR primer SEQ ID : 30(antisense) binding site

<220>
<221> primer_bind
<222> (1261)..(1296)
<223> PCR primer SEQ ID : 31 binding site

<220>
<221> primer_bind
<222> (1957)..(1980)
<223> PCR primer SEQ ID : 28(antisense) binding site

<220>
<221> sig_peptide
<222> (1)..(66)
<223> signal peptide

<400> 11

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atg gcg ccc gtc gcc gtc tgg gcc gcg ctg gcc gtc gga ctg gag ctc          48
Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu
  1               5                  10                  15

tgg gct gcg gcg cac gcc ttg ccc gcc cag gtg gca ttt aca ccc tac          96
Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
             20                  25                  30

gcc ccg gag ccc ggg agc aca tgc cgg ctc aga gaa tac tat gac cag         144
Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
         35                  40                  45

aca gct cag atg tgc tgc agc aaa tgc tcg ccg ggc caa cat gca aaa         192
Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
     50                  55                  60
```

Sequence Listing

```
gtc ttc tgt acc aag acc tcg gac acc gtg tgt gac tcc tgt gag gac      240
Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
 65                  70                  75                  80

agc aca tac acc cag ctc tgg aac tgg gtt ccc gag tgc ttg agc tgt      288
Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
                 85                  90                  95

ggc tcc cgc tgt agc tct gac cag gtg gaa act caa gcc tgc act cgg      336
Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
            100                 105                 110

gaa cag aac cgc atc tgc acc tgc agg ccc ggc tgg tac tgc gcg ctg      384
Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu
        115                 120                 125

agc aag cag gag ggg tgc cgg ctg tgc gcg ccg ctg cgc aag tgc cgc      432
Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
    130                 135                 140

ccg ggc ttc ggc gtg gcc aga cca gga act gaa aca tca gac gtg gtg      480
Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val
145                 150                 155                 160

tgc aag ccc tgt gcc ccg ggg acg ttc tcc aac acg act tca tcc acg      528
Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr
                165                 170                 175

gat att tgc agg ccc cac cag atc tgt aac gtg gtg gcc atc cct ggg      576
Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly
            180                 185                 190

aat gca agc atg gat gca aac tgc acg tcc ccg gag ccc aac agc aca      624
Asn Ala Ser Met Asp Ala Asn Cys Thr Ser Pro Glu Pro Asn Ser Thr
        195                 200                 205

tgc cgg ctc aga gaa tac tat gac cag aca gct cag atg tgc tgc agc      672
Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys Ser
    210                 215                 220
```

Sequence Listing

```
aaa tgc tcg ccg ggc caa cat gca aaa gtc ttc tgt acc aag acc tcg          720
Lys Cys Ser Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr Ser
225                 230                 235                 240

gac acc gtg tgt gac tcc tgt gag gac agc aca tac acc cag ctc tgg          768
Asp Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu Trp
                245                 250                 255

aac tgg gtt ccc gag tgc ttg agc tgt ggc tcc cgc tgt agc tct gac          816
Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser Asp
            260                 265                 270

cag gtg gaa act caa gcc tgc act cgg gaa cag aac cgc atc tgc acc          864
Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys Thr
        275                 280                 285

tgc agg ccc ggc tgg tac tgc gcg ctg agc aag cag gag ggg tgc cgg          912
Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys Arg
    290                 295                 300

ctg tgc gcg ccg ctg cgc aag tgc cgc ccg ggc ttc ggc gtg gcc aga          960
Leu Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala Arg
305                 310                 315                 320

cca gga act gaa aca tca gac gtg gtg tgc aag ccc tgt gcc ccg ggg         1008
Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro Gly
                325                 330                 335

acg ttc tcc aac acg act tca tcc acg gat att tgc agg ccc cac cag         1056
Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His Gln
            340                 345                 350

atc tgt aac gtg gtg gcc atc cct ggg aat gca agc atg gat gca gtc         1104
Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Met Asp Ala Val
        355                 360                 365

tgc acg tcc acg tcc ccc acc cgg agt atg gcc cca ggg gca gta cac         1152
Cys Thr Ser Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val His
    370                 375                 380
```

Sequence Listing

tta ccc cag cca gtg tcc aca cga tcc caa cac acg cag cca act cca 1200
Leu Pro Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr Pro
385 390 395 400

gaa ccc agc act gct cca agc acc tcc ttc ctg ctc cca atg ggc ccc 1248
Glu Pro Ser Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly Pro
405 410 415

agc ccc cca gct gaa ggg agc act ggc gac gca gag ccc aaa tct tgt 1296
Ser Pro Pro Ala Glu Gly Ser Thr Gly Asp Ala Glu Pro Lys Ser Cys
420 425 430

gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc ctg ggg 1344
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
435 440 445

gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc ctc atg 1392
Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
450 455 460

atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg agc cac 1440
Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
465 470 475 480

gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg gag gtg 1488
Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
485 490 495

cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc acg tac 1536
His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
500 505 510

cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg aat ggc 1584
Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
515 520 525

aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc ccc atc 1632
Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
530 535 540

Sequence Listing

gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca cag gtg 1680
Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
545 550 555 560

tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag gtc agc 1728
Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
565 570 575

ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag 1776
Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
580 585 590

tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct ccc 1824
Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
595 600 605

gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc acc gtg 1872
Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
610 615 620

gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg 1920
Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
625 630 635 640

cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc ctg tct 1968
His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
645 650 655

ccg ggt aaa tga 1980
Pro Gly Lys

<210> 12
<211> 659
<212> PRT
<213> Homo sapiens

<400> 12
Met Ala Pro Val Ala Val Trp Ala Ala Leu Ala Val Gly Leu Glu Leu

Sequence Listing

1 5 10 15

Trp Ala Ala Ala His Ala Leu Pro Ala Gln Val Ala Phe Thr Pro Tyr
20 25 30

Ala Pro Glu Pro Gly Ser Thr Cys Arg Leu Arg Glu Tyr Tyr Asp Gln
35 40 45

Thr Ala Gln Met Cys Cys Ser Lys Cys Ser Pro Gly Gln His Ala Lys
50 55 60

Val Phe Cys Thr Lys Thr Ser Asp Thr Val Cys Asp Ser Cys Glu Asp
65 70 75 80

Ser Thr Tyr Thr Gln Leu Trp Asn Trp Val Pro Glu Cys Leu Ser Cys
85 90 95

Gly Ser Arg Cys Ser Ser Asp Gln Val Glu Thr Gln Ala Cys Thr Arg
100 105 110

Glu Gln Asn Arg Ile Cys Thr Cys Arg Pro Gly Trp Tyr Cys Ala Leu
115 120 125

Ser Lys Gln Glu Gly Cys Arg Leu Cys Ala Pro Leu Arg Lys Cys Arg
130 135 140

Pro Gly Phe Gly Val Ala Arg Pro Gly Thr Glu Thr Ser Asp Val Val
145 150 155 160

Cys Lys Pro Cys Ala Pro Gly Thr Phe Ser Asn Thr Thr Ser Ser Thr
165 170 175

Asp Ile Cys Arg Pro His Gln Ile Cys Asn Val Val Ala Ile Pro Gly
180 185 190

Asn Ala Ser Met Asp Ala Asn Cys Thr Ser Pro Glu Pro Asn Ser Thr
195 200 205

Cys Arg Leu Arg Glu Tyr Tyr Asp Gln Thr Ala Gln Met Cys Cys Ser
210 215 220

Sequence Listing

Lys Cys Ser Pro Gly Gln His Ala Lys Val Phe Cys Thr Lys Thr Ser
225 230 235 240

Asp Thr Val Cys Asp Ser Cys Glu Asp Ser Thr Tyr Thr Gln Leu Trp
245 250 255

Asn Trp Val Pro Glu Cys Leu Ser Cys Gly Ser Arg Cys Ser Ser Asp
260 265 270

Gln Val Glu Thr Gln Ala Cys Thr Arg Glu Gln Asn Arg Ile Cys Thr
275 280 285

Cys Arg Pro Gly Trp Tyr Cys Ala Leu Ser Lys Gln Glu Gly Cys Arg
290 295 300

Leu Cys Ala Pro Leu Arg Lys Cys Arg Pro Gly Phe Gly Val Ala Arg
305 310 315 320

Pro Gly Thr Glu Thr Ser Asp Val Val Cys Lys Pro Cys Ala Pro Gly
325 330 335

Thr Phe Ser Asn Thr Thr Ser Ser Thr Asp Ile Cys Arg Pro His Gln
340 345 350

Ile Cys Asn Val Val Ala Ile Pro Gly Asn Ala Ser Met Asp Ala Val
355 360 365

Cys Thr Ser Thr Ser Pro Thr Arg Ser Met Ala Pro Gly Ala Val His
370 375 380

Leu Pro Gln Pro Val Ser Thr Arg Ser Gln His Thr Gln Pro Thr Pro
385 390 395 400

Glu Pro Ser Thr Ala Pro Ser Thr Ser Phe Leu Leu Pro Met Gly Pro
405 410 415

Ser Pro Pro Ala Glu Gly Ser Thr Gly Asp Ala Glu Pro Lys Ser Cys
420 425 430

Sequence Listing

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
435 440 445

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
450 455 460

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
465 470 475 480

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
485 490 495

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
500 505 510

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
515 520 525

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
530 535 540

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
545 550 555 560

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
565 570 575

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
580 585 590

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
595 600 605

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
610 615 620

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
625 630 635 640

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser

Sequence Listing

645 650 655

Pro Gly Lys

<210> 13
<211> 1314
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1311)
<223> CD2-IgG

<220>
<221> C_region
<222> (613)..(1314)
<223> Hinge, CH2, CH3 region

<220>
<221> misc_signal
<222> (265)..(273)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (421)..(429)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (448)..(456)
<223> N-linked glycosylation site

Sequence Listing

<220>
<221> primer_bind
<222> (1)..(27)
<223> PCR primer SEQ ID : 40 binding site

<220>
<221> primer_bind
<222> (589)..(618)
<223> PCR primer SEQ ID : 41(antisense) binding site

<220>
<221> primer_bind
<222> (611)..(633)
<223> PCR primer SEQ ID : 42 binding site

<220>
<221> primer_bind
<222> (1292)..(1314)
<223> PCR primer SEQ ID : 28(antisense) binding site

<220>
<221> sig_peptide
<222> (1)..(72)
<223> signal peptide

<400> 13

```
atg agc ttt cca tgt aaa ttt gta gcc agc ttc ctt ctg att ttc aat          48
Met Ser Phe Pro Cys Lys Phe Val Ala Ser Phe Leu Leu Ile Phe Asn
  1               5                  10                  15

gtt tct tcc aaa ggt gca gtc tcc aaa gag att acg aat gcc ttg gaa          96
Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn Ala Leu Glu
             20                  25                  30
```

Sequence Listing

```
acc tgg ggt gcc ttg ggt cag gac atc aac ttg gac att cct agt ttt          144
Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser Phe
         35                  40                  45

caa atg agt gat gat att gac gat ata aaa tgg gaa aaa act tca gac          192
Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp
     50                  55                  60

aag aaa aag att gca caa ttc aga aaa gag aaa gag act ttc aag gaa          240
Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu
 65                  70                  75                  80

aaa gat aca tat aag cta ttt aaa aat gga act ctg aaa att aag cat          288
Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His
                 85                  90                  95

ctg aag acc gat gat cag gat atc tac aag gta tca ata tat gat aca          336
Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr
            100                 105                 110

aaa gga aaa aat gtg ttg gaa aaa ata ttt gat ttg aag att caa gag          384
Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu Lys Ile Gln Glu
        115                 120                 125

agg gtc tca aaa cca aag atc tcc tgg act tgt atc aac aca acc ctg          432
Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu
    130                 135                 140

acc tgt gag gta atg aat gga act gac ccc gaa tta aac ctg tat caa          480
Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu Asn Leu Tyr Gln
145                 150                 155                 160

gat ggg aaa cat cta aaa ctt tct cag agg gtc atc aca cac aag tgg          528
Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr His Lys Trp
                165                 170                 175

acc acc agc ctg agt gca aaa ttc aag tgc aca gca ggg aac aaa gtc          576
Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys Val
            180                 185                 190
```

Sequence Listing

```
agc aag gaa tcc agt gtc gag cct gtc agc tgt cct gca gag ccc aaa          624
Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro Ala Glu Pro Lys
        195                 200                 205

tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa ctc          672
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
    210                 215                 220

ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac acc          720
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
225                 230                 235                 240

ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac gtg          768
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
                245                 250                 255

agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc gtg          816
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
            260                 265                 270

gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac agc          864
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
        275                 280                 285

acg tac cgg gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg          912
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
    290                 295                 300

aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca gcc          960
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
305                 310                 315                 320

ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa cca         1008
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
                325                 330                 335

cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac cag         1056
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
            340                 345                 350
```

Sequence Listing

```
gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc gcc        1104
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
        355                 360                 365

gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg        1152
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
    370                 375                 380

cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag ctc        1200
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
385                 390                 395                 400

acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc tcc        1248
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
                405                 410                 415

gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc tcc        1296
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
            420                 425                 430

ctg tct ccg ggt aaa    tga                                          1314
Leu Ser Pro Gly Lys
        435
```

<210> 14
<211> 437
<212> PRT
<213> Homo sapiens

<400> 14

```
Met Ser Phe Pro Cys Lys Phe Val Ala Ser Phe Leu Leu Ile Phe Asn
  1               5                  10                  15

Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn Ala Leu Glu
            20                  25                  30

Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser Phe
        35                  40                  45
```

Sequence Listing

Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp
50 55 60

Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu
65 70 75 80

Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His
85 90 95

Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr
100 105 110

Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu Lys Ile Gln Glu
115 120 125

Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu
130 135 140

Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu Asn Leu Tyr Gln
145 150 155 160

Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr His Lys Trp
165 170 175

Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys Val
180 185 190

Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro Ala Glu Pro Lys
195 200 205

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
210 215 220

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
225 230 235 240

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
245 250 255

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val

Sequence Listing

260 265 270

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
275 280 285

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
290 295 300

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
305 310 315 320

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
325 330 335

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
340 345 350

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
355 360 365

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
370 375 380

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
385 390 395 400

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
405 410 415

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
420 425 430

Leu Ser Pro Gly Lys
435

<210> 15
<211> 1134
<212> DNA
<213> Homo sapiens

Sequence Listing

<220>
<221> CDS
<222> (1)..(1131)
<223> CTLA4-IgG

<220>
<221> C_region
<222> (433)..(1134)
<223> Hinge, CH2, CH3 region

<220>
<221> misc_signal
<222> (289)..(297)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (385)..(393)
<223> N-linked glycosylation site

<220>
<221> primer_bind
<222> (1)..(15)
<223> PCR primer SEQ ID : 43 binding site

<220>
<221> primer_bind
<222> (409)..(438)
<223> PCR primer SEQ ID : 44(antisense) binding site

<220>
<221> primer_bind
<222> (430)..(453)

Sequence Listing

<223> PCR primer SEQ ID : 42 binding site

<220>
<221> primer_bind
<222> (1111)..(1134)
<223> PCR primer SEQ ID : 28(antisense) binding site

<220>
<221> sig_peptide
<222> (1)..(63)
<223> signal peptide

<400> 15

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atg agg acc tgg ccc tgc act ctc ctg ttt ttt ctt ctc ttc atc cct          48
Met Arg Thr Trp Pro Cys Thr Leu Leu Phe Phe Leu Leu Phe Ile Pro
  1               5                  10                  15

gtc ttc tgc aaa gca atg cac gtg gcc cag cct gct gtg gta ctg gcc          96
Val Phe Cys Lys Ala Met His Val Ala Gln Pro Ala Val Val Leu Ala
             20                  25                  30

agc agc cga ggc atc gcc agc ttt gtg tgt gag tat gca tct cca ggc         144
Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly
         35                  40                  45

aaa gcc act gag gtc cgg gtg aca gtg ctt cgg cag gct gac agc cag         192
Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln
     50                  55                  60

gtg act gaa gtc tgt gcg gca acc tac atg atg ggg aat gag ttg acc         240
Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr
 65                  70                  75                  80

ttc cta gat gat tcc atc tgc acg ggc acc tcc agt gga aat caa gtg         288
Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val
                 85                  90                  95
```

Sequence Listing

```
aac ctc act atc caa gga ctg agg gcc atg gac acg gga ctc tac atc          336
Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
            100                 105                 110

tgc aag gtg gag ctc atg tac cca ccg cca tac tac ctg ggc ata ggc          384
Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly
        115                 120                 125

aac gga acc cag att tat gta att gat cca gaa ccg tgc cca gat tct          432
Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser
    130                 135                 140

gca gag ccc aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca          480
Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
145                 150                 155                 160

gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa          528
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
                165                 170                 175

ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg          576
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
            180                 185                 190

gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac          624
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
        195                 200                 205

gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag          672
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
    210                 215                 220

cag tac aac agc acg tac cgg gtg gtc agc gtc ctc acc gtc ctg cac          720
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
225                 230                 235                 240

cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa          768
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                245                 250                 255
```

Sequence Listing

gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag 816
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
260 265 270

ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg 864
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
275 280 285

acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc 912
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
290 295 300

agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac 960
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
305 310 315 320

tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc 1008
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
325 330 335

tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc 1056
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
340 345 350

ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag 1104
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
355 360 365

aag agc ctc tcc ctg tct ccg ggt aaa tga 1134
Lys Ser Leu Ser Leu Ser Pro Gly Lys
370 375

<210> 16
<211> 377
<212> PRT
<213> Homo sapiens

<400> 16
Met Arg Thr Trp Pro Cys Thr Leu Leu Phe Phe Leu Leu Phe Ile Pro

Sequence Listing

```
1               5                   10                  15

Val Phe Cys Lys Ala Met His Val Ala Gln Pro Ala Val Val Leu Ala
            20                  25                  30

Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly
        35                  40                  45

Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln
    50                  55                  60

Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr
65                  70                  75                  80

Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val
                85                  90                  95

Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
            100                 105                 110

Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly
        115                 120                 125

Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser
    130                 135                 140

Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
145                 150                 155                 160

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
                165                 170                 175

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
            180                 185                 190

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
        195                 200                 205

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
    210                 215                 220
```

Sequence Listing

```
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His
225                 230                 235                 240

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                245                 250                 255

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
            260                 265                 270

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
        275                 280                 285

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
    290                 295                 300

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
305                 310                 315                 320

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
                325                 330                 335

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
            340                 345                 350

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
        355                 360                 365

Lys Ser Leu Ser Leu Ser Pro Gly Lys
    370                 375
```

<210> 17
<211> 1854
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1851)

Sequence Listing

<223> CD2-CD2-IgG

<220>
<221> C_region
<222> (1153)..(1854)
<223> Hinge, CH2, CH3 region

<220>
<221> misc_signal
<222> (265)..(273)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (421)..(429)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (448)..(456)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (805)..(813)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (961)..(969)
<223> N-linked glycosylation site

<220>

Sequence Listing

<221> misc_signal
<222> (988)..(996)
<223> N-linked glycosylation site

<220>
<221> primer_bind
<222> (1)..(27)
<223> PCR primer SEQ ID : 40 binding site

<220>
<221> primer_bind
<222> (598)..(612)
<223> PCR primer SEQ ID : 46(antisense) binding site

<220>
<221> primer_bind
<222> (612)..(630)
<223> PCR primer SEQ ID : 45 binding site

<220>
<221> primer_bind
<222> (1128)..(1158)
<223> PCR primer SEQ ID : 41(antisense) binding site

<220>
<221> primer_bind
<222> (1151)..(1173)
<223> PCR primer SEQ ID : 42 binding site

<220>
<221> primer_bind
<222> (1832)..(1854)
<223> PCR primer SEQ ID : 28(antisense) binding site

Sequence Listing

<220>
<221> sig_peptide
<222> (1)..(72)
<223> signal peptide

<400> 17

```
atg agc ttt cca tgt aaa ttt gta gcc agc ttc ctt ctg att ttc aat      48
Met Ser Phe Pro Cys Lys Phe Val Ala Ser Phe Leu Leu Ile Phe Asn
  1               5                  10                  15

gtt tct tcc aaa ggt gca gtc tcc aaa gag att acg aat gcc ttg gaa      96
Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn Ala Leu Glu
             20                  25                  30

acc tgg ggt gcc ttg ggt cag gac atc aac ttg gac att cct agt ttt     144
Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser Phe
         35                  40                  45

caa atg agt gat gat att gac gat ata aaa tgg gaa aaa act tca gac     192
Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp
     50                  55                  60

aag aaa aag att gca caa ttc aga aaa gag aaa gag act ttc aag gaa     240
Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu
 65                  70                  75                  80

aaa gat aca tat aag cta ttt aaa aat gga act ctg aaa att aag cat     288
Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His
                 85                  90                  95

ctg aag acc gat gat cag gat atc tac aag gta tca ata tat gat aca     336
Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr
            100                 105                 110

aaa gga aaa aat gtg ttg gaa aaa ata ttt gat ttg aag att caa gag     384
Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu Lys Ile Gln Glu
        115                 120                 125
```

Sequence Listing

```
agg gtc tca aaa cca aag atc tcc tgg act tgt atc aac aca acc ctg          432
Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu
    130                 135                 140

acc tgt gag gta atg aat gga act gac ccc gaa tta aac ctg tat caa          480
Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu Asn Leu Tyr Gln
145                 150                 155                 160

gat ggg aaa cat cta aaa ctt tct cag agg gtc atc aca cac aag tgg          528
Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr His Lys Trp
                165                 170                 175

acc acc agc ctg agt gca aaa ttc aag tgc aca gca ggg aac aaa gtc          576
Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys Val
            180                 185                 190

agc aag gaa tcc agt gtc gag cct gtc agc tgt cct aaa gag att acg          624
Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro Lys Glu Ile Thr
        195                 200                 205

aat gcc ttg gaa acc tgg ggt gcc ttg ggt cag gac atc aac ttg gac          672
Asn Ala Leu Glu Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp
    210                 215                 220

att cct agt ttt caa atg agt gat gat att gac gat ata aaa tgg gaa          720
Ile Pro Ser Phe Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu
225                 230                 235                 240

aaa act tca gac aag aaa aag att gca caa ttc aga aaa gag aaa gag          768
Lys Thr Ser Asp Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu
                245                 250                 255

act ttc aag gaa aaa gat aca tat aag cta ttt aaa aat gga act ctg          816
Thr Phe Lys Glu Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu
            260                 265                 270

aaa att aag cat ctg aag acc gat gat cag gat atc tac aag gta tca          864
Lys Ile Lys His Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser
        275                 280                 285
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Sequence Listing

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ata tat gat aca aaa gga aaa aat gtg ttg gaa aaa ata ttt gat ttg          912
Ile Tyr Asp Thr Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu
    290                 295                 300

aag att caa gag agg gtc tca aaa cca aag atc tcc tgg act tgt atc          960
Lys Ile Gln Glu Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile
305                 310                 315                 320

aac aca acc ctg acc tgt gag gta atg aat gga act gac ccc gaa tta         1008
Asn Thr Thr Leu Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu
                325                 330                 335

aac ctg tat caa gat ggg aaa cat cta aaa ctt tct cag agg gtc atc         1056
Asn Leu Tyr Gln Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile
            340                 345                 350

aca cac aag tgg acc acc agc ctg agt gca aaa ttc aag tgc aca gca         1104
Thr His Lys Trp Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala
        355                 360                 365

ggg aac aaa gtc agc aag gaa tcc agt gtc gag cct gtc agc tgt cct         1152
Gly Asn Lys Val Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro
    370                 375                 380

gca gag ccc aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca         1200
Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
385                 390                 395                 400

gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa         1248
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
                405                 410                 415

ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg         1296
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
            420                 425                 430

gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac         1344
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
        435                 440                 445
```

Sequence Listing

gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag 1392
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
450 455 460

cag tac aac agc acg tac cgg gtg gtc agc gtc ctc acc gtc tgt cac 1440
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His
465 470 475 480

cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa 1488
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
485 490 495

gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag 1536
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
500 505 510

ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg 1584
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
515 520 525

acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc 1632
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
530 535 540

agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac 1680
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
545 550 555 560

tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc 1728
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
565 570 575

tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc 1776
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
580 585 590

ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag 1824
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
595 600 605

Sequence Listing

aag agc ctc tcc ctg tct ccg ggt aaa tga 1854
Lys Ser Leu Ser Leu Ser Pro Gly Lys
610 615

<210> 18
<211> 617
<212> PRT
<213> Homo sapiens

<400> 18
Met Ser Phe Pro Cys Lys Phe Val Ala Ser Phe Leu Leu Ile Phe Asn
1 5 10 15

Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn Ala Leu Glu
20 25 30

Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser Phe
35 40 45

Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp
50 55 60

Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu
65 70 75 80

Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His
85 90 95

Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr
100 105 110

Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu Lys Ile Gln Glu
115 120 125

Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu
130 135 140

Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu Asn Leu Tyr Gln
145 150 155 160

Sequence Listing

Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr His Lys Trp
165 170 175

Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys Val
180 185 190

Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro Lys Glu Ile Thr
195 200 205

Asn Ala Leu Glu Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp
210 215 220

Ile Pro Ser Phe Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu
225 230 235 240

Lys Thr Ser Asp Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu
245 250 255

Thr Phe Lys Glu Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu
260 265 270

Lys Ile Lys His Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser
275 280 285

Ile Tyr Asp Thr Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu
290 295 300

Lys Ile Gln Glu Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile
305 310 315 320

Asn Thr Thr Leu Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu
325 330 335

Asn Leu Tyr Gln Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile
340 345 350

Thr His Lys Trp Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala
355 360 365

Sequence Listing

Gly Asn Lys Val Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro
370 375 380

Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
385 390 395 400

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
405 410 415

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
420 425 430

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
435 440 445

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
450 455 460

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His
465 470 475 480

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
485 490 495

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
500 505 510

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
515 520 525

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
530 535 540

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
545 550 555 560

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
565 570 575

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val

Sequence Listing

580 585 590

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
595 600 605

Lys Ser Leu Ser Leu Ser Pro Gly Lys
610 615

<210> 19
<211> 1509
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1506)
<223> CTLA4-CTLA4-IgG

<220>
<221> C_region
<222> (808)..(1509)
<223> Hinge, CH2, CH3 region

<220>
<221> misc_signal
<222> (289)..(297)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (385)..(393)
<223> N-linked glycosylation site

<220>
<221> misc_signal

Sequence Listing

<222> (664)..(672)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (760)..(768)
<223> N-linked glycosylation site

<220>
<221> primer_bind
<222> (1)..(15)
<223> PCR primer SEQ ID : 43 binding site

<220>
<221> primer_bind
<222> (418)..(431)
<223> PCR primer SEQ ID : 48(antisense) binding site

<220>
<221> primer_bind
<222> (432)..(453)
<223> PCR primer SEQ ID : 47 binding site

<220>
<221> primer_bind
<222> (784)..(813)
<223> PCR primer SEQ ID : 44(antisense) binding site

<220>
<221> primer_bind
<222> (805)..(826)
<223> PCR primer SEQ ID : 42 binding site

Sequence Listing

<220>
<221> primer_bind
<222> (1486)..(1509)
<223> PCR primer SEQ ID : 28(antisense) binding site

<220>
<221> sig_peptide
<222> (1)..(63)
<223> signal peptide

<400> 19

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atg agg acc tgg ccc tgc act ctc ctg ttt ttt ctt ctc ttc atc cct          48
Met Arg Thr Trp Pro Cys Thr Leu Leu Phe Phe Leu Leu Phe Ile Pro
  1               5                  10                  15

gtc ttc tgc aaa gca atg cac gtg gcc cag cct gct gtg gta ctg gcc          96
Val Phe Cys Lys Ala Met His Val Ala Gln Pro Ala Val Val Leu Ala
             20                  25                  30

agc agc cga ggc atc gcc agc ttt gtg tgt gag tat gca tct cca ggc         144
Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly
         35                  40                  45

aaa gcc act gag gtc cgg gtg aca gtg ctt cgg cag gct gac agc cag         192
Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln
     50                  55                  60

gtg act gaa gtc tgt gcg gca acc tac atg atg ggg aat gag ttg acc         240
Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr
 65                  70                  75                  80

ttc cta gat gat tcc atc tgc acg ggc acc tcc agt gga aat caa gtg         288
Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val
                 85                  90                  95

aac ctc act atc caa gga ctg agg gcc atg gac acg gga ctc tac atc         336
Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
            100                 105                 110
```

Sequence Listing

```
tgc aag gtg gag ctc atg tac cca ccg cca tac tac ctg ggc ata ggc          384
Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly
        115                 120                 125

aac gga acc cag att tat gta att gat cca gaa ccg tgc cca gat tcg          432
Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser
    130                 135                 140

gat aac atg cac gtg gcc cag cct gct gtg gta ctg gcc agc agc cga          480
Asp Asn Met His Val Ala Gln Pro Ala Val Val Leu Ala Ser Ser Arg
145                 150                 155                 160

ggc atc gcc agc ttt gtg tgt gag tat gca tct cca ggc aaa gcc act          528
Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly Lys Ala Thr
                165                 170                 175

gag gtc cgg gtg aca gtg ctt cgg cag gct gac agc cag gtg act gaa          576
Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln Val Thr Glu
            180                 185                 190

gtc tgt gcg gca acc tac atg atg ggg aat gag ttg acc ttc cta gat          624
Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr Phe Leu Asp
        195                 200                 205

gat tcc atc tgc acg ggc acc tcc agt gga aat caa gtg aac ctc act          672
Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val Asn Leu Thr
    210                 215                 220

atc caa gga ctg agg gcc atg gac acg gga ctc tac atc tgc aag gtg          720
Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile Cys Lys Val
225                 230                 235                 240

gag ctc atg tac cca ccg cca tac tac ctg ggc ata ggc aac gga acc          768
Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly Asn Gly Thr
                245                 250                 255

cag att tat gta att gat cca gaa ccg tgc cca gat tct gca gag ccc          816
Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser Ala Glu Pro
            260                 265                 270
```

Sequence Listing

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aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa          864
Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
        275                 280                 285

ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac          912
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
    290                 295                 300

acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac          960
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
305                 310                 315                 320

gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc         1008
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
                325                 330                 335

gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac         1056
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
            340                 345                 350

agc acg tac cgg gtg gtc agc gtc ctc acc gtc tgt cac cag gac tgg         1104
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His Gln Asp Trp
        355                 360                 365

ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca         1152
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
    370                 375                 380

gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa         1200
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
385                 390                 395                 400

cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac         1248
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
                405                 410                 415

cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc         1296
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
            420                 425                 430
```

Sequence Listing

gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc 1344
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
435 440 445

acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag 1392
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
450 455 460

ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc 1440
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
465 470 475 480

tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc 1488
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
485 490 495

tcc ctg tct ccg ggt aaa tga 1509
Ser Leu Ser Pro Gly Lys
500

<210> 20
<211> 502
<212> PRT
<213> Homo sapiens

<400> 20
Met Arg Thr Trp Pro Cys Thr Leu Leu Phe Phe Leu Leu Phe Ile Pro
1 5 10 15

Val Phe Cys Lys Ala Met His Val Ala Gln Pro Ala Val Val Leu Ala
20 25 30

Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly
35 40 45

Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln
50 55 60

Sequence Listing

Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr
65 70 75 80

Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val
85 90 95

Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
100 105 110

Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly
115 120 125

Asn Gly Thr Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser
130 135 140

Asp Asn Met His Val Ala Gln Pro Ala Val Val Leu Ala Ser Ser Arg
145 150 155 160

Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly Lys Ala Thr
165 170 175

Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln Val Thr Glu
180 185 190

Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr Phe Leu Asp
195 200 205

Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val Asn Leu Thr
210 215 220

Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile Cys Lys Val
225 230 235 240

Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly Asn Gly Thr
245 250 255

Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser Ala Glu Pro
260 265 270

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu

Sequence Listing

275 280 285

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
290 295 300

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
305 310 315 320

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
325 330 335

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
340 345 350

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His Gln Asp Trp
355 360 365

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
370 375 380

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
385 390 395 400

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
405 410 415

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
420 425 430

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
435 440 445

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
450 455 460

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
465 470 475 480

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
485 490 495

Sequence Listing

Ser Leu Ser Pro Gly Lys
500

<210> 21
<211> 1854
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(1851)
<223> mgCD2-CD2-IgG

<220>
<221> C_region
<222> (1153)..(1854)
<223> Hinge, CH2, CH3 region

<220>
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<222> (265)..(273)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (421)..(429)
<223> N-linked glycosylation site

<220>
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<222> (448)..(456)
<223> N-linked glycosylation site

Sequence Listing

<220>
<221> misc_signal
<222> (598)..(606)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (616)..(624)
<223> N-linked glycosylation site

<220>
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<222> (805)..(813)
<223> N-linked glycosylation site

<220>
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<222> (961)..(969)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (988)..(996)
<223> N-linked glycosylation site

<220>
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<222> (1)..(27)
<223> PCR primer SEQ ID : 40 binding site

<220>
<221> primer_bind
<222> (588)..(630)
<223> PCR primer SEQ ID : 50(antisense) binding site

Sequence Listing

<220>
<221> primer_bind
<222> (588)..(630)
<223> PCR primer SEQ ID : 49 binding site

<220>
<221> primer_bind
<222> (1128)..(1158)
<223> PCR primer SEQ ID : 41(antisense) binding site

<220>
<221> primer_bind
<222> (1151)..(1173)
<223> PCR primer SEQ ID : 42 binding site

<220>
<221> primer_bind
<222> (1832)..(1854)
<223> PCR primer SEQ ID : 28(antisense) binding site

<220>
<221> sig_peptide
<222> (1)..(72)
<223> signal peptide

<400> 21

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atg agc ttt cca tgt aaa ttt gta gcc agc ttc ctt ctg att ttc aat          48
Met Ser Phe Pro Cys Lys Phe Val Ala Ser Phe Leu Leu Ile Phe Asn
  1               5                  10                  15

gtt tct tcc aaa ggt gca gtc tcc aaa gag att acg aat gcc ttg gaa          96
Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn Ala Leu Glu
             20                  25                  30
```

Sequence Listing

```
acc tgg ggt gcc ttg ggt cag gac atc aac ttg gac att cct agt ttt   144
Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser Phe
         35                  40                  45

caa atg agt gat gat att gac gat ata aaa tgg gaa aaa act tca gac   192
Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp
     50                  55                  60

aag aaa aag att gca caa ttc aga aaa gag aaa gag act ttc aag gaa   240
Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu
 65                  70                  75                  80

aaa gat aca tat aag cta ttt aaa aat gga act ctg aaa att aag cat   288
Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His
                 85                  90                  95

ctg aag acc gat gat cag gat atc tac aag gta tca ata tat gat aca   336
Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr
            100                 105                 110

aaa gga aaa aat gtg ttg gaa aaa ata ttt gat ttg aag att caa gag   384
Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu Lys Ile Gln Glu
        115                 120                 125

agg gtc tca aaa cca aag atc tcc tgg act tgt atc aac aca acc ctg   432
Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu
    130                 135                 140

acc tgt gag gta atg aat gga act gac ccc gaa tta aac ctg tat caa   480
Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu Asn Leu Tyr Gln
145                 150                 155                 160

gat ggg aaa cat cta aaa ctt tct cag agg gtc atc aca cac aag tgg   528
Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr His Lys Trp
                165                 170                 175

acc acc agc ctg agt gca aaa ttc aag tgc aca gca ggg aac aaa gtc   576
Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys Val
            180                 185                 190
```

Sequence Listing

```
agc aag gaa tcc agt gtc gag aat gtc agc tgt cct aaa aat att acg      624
Ser Lys Glu Ser Ser Val Glu Asn Val Ser Cys Pro Lys Asn Ile Thr
        195                 200                 205

aat gcc ttg gaa acc tgg ggt gcc ttg ggt cag gac atc aac ttg gac      672
Asn Ala Leu Glu Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp
    210                 215                 220

att cct agt ttt caa atg agt gat gat att gac gat ata aaa tgg gaa      720
Ile Pro Ser Phe Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu
225                 230                 235                 240

aaa act tca gac aag aaa aag att gca caa ttc aga aaa gag aaa gag      768
Lys Thr Ser Asp Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu
                245                 250                 255

act ttc aag gaa aaa gat aca tat aag cta ttt aaa aat gga act ctg      816
Thr Phe Lys Glu Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu
            260                 265                 270

aaa att aag cat ctg aag acc gat gat cag gat atc tac aag gta tca      864
Lys Ile Lys His Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser
        275                 280                 285

ata tat gat aca aaa gga aaa aat gtg ttg gaa aaa ata ttt gat ttg      912
Ile Tyr Asp Thr Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu
    290                 295                 300

aag att caa gag agg gtc tca aaa cca aag atc tcc tgg act tgt atc      960
Lys Ile Gln Glu Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile
305                 310                 315                 320

aac aca acc ctg acc tgt gag gta atg aat gga act gac ccc gaa tta     1008
Asn Thr Thr Leu Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu
                325                 330                 335

aac ctg tat caa gat ggg aaa cat cta aaa ctt tct cag agg gtc atc     1056
Asn Leu Tyr Gln Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile
            340                 345                 350
```

Sequence Listing

```
aca cac aag tgg acc acc agc ctg agt gca aaa ttc aag tgc aca gca        1104
Thr His Lys Trp Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala
        355                 360                 365

ggg aac aaa gtc agc aag gaa tcc agt gtc gag cct gtc agc tgt cct        1152
Gly Asn Lys Val Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro
    370                 375                 380

gca gag ccc aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca        1200
Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
385                 390                 395                 400

gca cct gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa        1248
Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
                405                 410                 415

ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg        1296
Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
            420                 425                 430

gtg gtg gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac        1344
Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
        435                 440                 445

gtg gac ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag        1392
Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
    450                 455                 460

cag tac aac agc acg tac cgg gtg gtc agc gtc ctc acc gtc tgt cac        1440
Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His
465                 470                 475                 480

cag gac tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa        1488
Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
                485                 490                 495

gcc ctc cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag        1536
Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
            500                 505                 510
```

Sequence Listing

```
ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg        1584
Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
        515                 520                 525

acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc        1632
Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
    530                 535                 540

agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac        1680
Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
545                 550                 555                 560

tac aag acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc        1728
Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
                565                 570                 575

tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc        1776
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
            580                 585                 590

ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag        1824
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
        595                 600                 605

aag agc ctc tcc ctg tct ccg ggt aaa          tga                       1854
Lys Ser Leu Ser Leu Ser Pro Gly Lys
    610                 615
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<210> 22
<211> 617
<212> PRT
<213> Homo sapiens

<400> 22

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Met Ser Phe Pro Cys Lys Phe Val Ala Ser Phe Leu Leu Ile Phe Asn
  1               5                  10                  15

Val Ser Ser Lys Gly Ala Val Ser Lys Glu Ile Thr Asn Ala Leu Glu
```

Sequence Listing

20 25 30

Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp Ile Pro Ser Phe
35 40 45

Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu Lys Thr Ser Asp
50 55 60

Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu Thr Phe Lys Glu
65 70 75 80

Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu Lys Ile Lys His
85 90 95

Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser Ile Tyr Asp Thr
100 105 110

Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu Lys Ile Gln Glu
115 120 125

Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile Asn Thr Thr Leu
130 135 140

Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu Asn Leu Tyr Gln
145 150 155 160

Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile Thr His Lys Trp
165 170 175

Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala Gly Asn Lys Val
180 185 190

Ser Lys Glu Ser Ser Val Glu Asn Val Ser Cys Pro Lys Asn Ile Thr
195 200 205

Asn Ala Leu Glu Thr Trp Gly Ala Leu Gly Gln Asp Ile Asn Leu Asp
210 215 220

Ile Pro Ser Phe Gln Met Ser Asp Asp Ile Asp Asp Ile Lys Trp Glu
225 230 235 240

Sequence Listing

Lys Thr Ser Asp Lys Lys Lys Ile Ala Gln Phe Arg Lys Glu Lys Glu
245 250 255

Thr Phe Lys Glu Lys Asp Thr Tyr Lys Leu Phe Lys Asn Gly Thr Leu
260 265 270

Lys Ile Lys His Leu Lys Thr Asp Asp Gln Asp Ile Tyr Lys Val Ser
275 280 285

Ile Tyr Asp Thr Lys Gly Lys Asn Val Leu Glu Lys Ile Phe Asp Leu
290 295 300

Lys Ile Gln Glu Arg Val Ser Lys Pro Lys Ile Ser Trp Thr Cys Ile
305 310 315 320

Asn Thr Thr Leu Thr Cys Glu Val Met Asn Gly Thr Asp Pro Glu Leu
325 330 335

Asn Leu Tyr Gln Asp Gly Lys His Leu Lys Leu Ser Gln Arg Val Ile
340 345 350

Thr His Lys Trp Thr Thr Ser Leu Ser Ala Lys Phe Lys Cys Thr Ala
355 360 365

Gly Asn Lys Val Ser Lys Glu Ser Ser Val Glu Pro Val Ser Cys Pro
370 375 380

Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro
385 390 395 400

Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys
405 410 415

Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val
420 425 430

Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr
435 440 445

Sequence Listing

Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu
450 455 460

Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His
465 470 475 480

Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys
485 490 495

Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln
500 505 510

Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu
515 520 525

Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro
530 535 540

Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn
545 550 555 560

Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu
565 570 575

Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val
580 585 590

Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln
595 600 605

Lys Ser Leu Ser Leu Ser Pro Gly Lys
610 615

<210> 23
<211> 1509
<212> DNA
<213> Homo sapiens

<220>

Sequence Listing

<221> CDS
<222> (1)..(1506)
<223> mgCTLA4-CTLA4-IgG

<220>
<221> C_region
<222> (808)..(1509)
<223> Hinge, CH2, CH3 region

<220>
<221> misc_signal
<222> (289)..(297)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (385)..(393)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (403)..(411)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (424)..(432)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (439)..(447)
<223> N-linked glycosylation site

Sequence Listing

<220>
<221> misc_signal
<222> (664)..(672)
<223> N-linked glycosylation site

<220>
<221> misc_signal
<222> (760)..(768)
<223> N-linked glycosylation site

<220>
<221> primer_bind
<222> (1)..(15)
<223> PCR primer SEQ ID : 43 binding site

<220>
<221> primer_bind
<222> (394)..(456)
<223> PCR primer SEQ ID : 52(antisense) binding site

<220>
<221> primer_bind
<222> (397)..(460)
<223> PCR primer SEQ ID : 51 binding site

<220>
<221> primer_bind
<222> (784)..(813)
<223> PCR primer SEQ ID : 44(antisense) binding site

<220>
<221> primer_bind
<222> (805)..(826)

Sequence Listing

<223> PCR primer SEQ ID : 42 binding site

<220>
<221> primer_bind
<222> (1486)..(1509)
<223> PCR primer SEQ ID : 28(antisense) binding site

<220>
<221> sig_peptide
<222> (1)..(63)
<223> signal peptide

<400> 23

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atg agg acc tgg ccc tgc act ctc ctg ttt ttt ctt ctc ttc atc cct          48
Met Arg Thr Trp Pro Cys Thr Leu Leu Phe Phe Leu Leu Phe Ile Pro
  1               5                  10                  15

gtc ttc tgc aaa gca atg cac gtg gcc cag cct gct gtg gta ctg gcc          96
Val Phe Cys Lys Ala Met His Val Ala Gln Pro Ala Val Val Leu Ala
             20                  25                  30

agc agc cga ggc atc gcc agc ttt gtg tgt gag tat gca tct cca ggc         144
Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly
         35                  40                  45

aaa gcc act gag gtc cgg gtg aca gtg ctt cgg cag gct gac agc cag         192
Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln
     50                  55                  60

gtg act gaa gtc tgt gcg gca acc tac atg atg ggg aat gag ttg acc         240
Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr
 65                  70                  75                  80

ttc cta gat gat tcc atc tgc acg ggc acc tcc agt gga aat caa gtg         288
Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val
                 85                  90                  95
```

Sequence Listing

```
aac ctc act atc caa gga ctg agg gcc atg gac acg gga ctc tac atc          336
Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
            100                 105                 110

tgc aag gtg gag ctc atg tac cca ccg cca tac tac ctg ggc ata ggc          384
Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly
        115                 120                 125

aac gga acc cag att tat gta aat gat aca gaa ccg tgc aat gat tcg          432
Asn Gly Thr Gln Ile Tyr Val Asn Asp Thr Glu Pro Cys Asn Asp Ser
    130                 135                 140

gat aac aat cac acg gcc cag cct gct gtg gta ctg gcc agc agc cga          480
Asp Asn Asn His Thr Ala Gln Pro Ala Val Val Leu Ala Ser Ser Arg
145                 150                 155                 160

ggc atc gcc agc ttt gtg tgt gag tat gca tct cca ggc aaa gcc act          528
Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly Lys Ala Thr
                165                 170                 175

gag gtc cgg gtg aca gtg ctt cgg cag gct gac agc cag gtg act gaa          576
Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln Val Thr Glu
            180                 185                 190

gtc tgt gcg gca acc tac atg atg ggg aat gag ttg acc ttc cta gat          624
Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr Phe Leu Asp
        195                 200                 205

gat tcc atc tgc acg ggc acc tcc agt gga aat caa gtg aac ctc act          672
Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val Asn Leu Thr
    210                 215                 220

atc caa gga ctg agg gcc atg gac acg gga ctc tac atc tgc aag gtg          720
Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile Cys Lys Val
225                 230                 235                 240

gag ctc atg tac cca ccg cca tac tac ctg ggc ata ggc aac gga acc          768
Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly Asn Gly Thr
                245                 250                 255
```

Sequence Listing

```
cag att tat gta att gat cca gaa ccg tgc cca gat tct gca gag ccc          816
Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser Ala Glu Pro
            260                 265                 270

aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct gaa          864
Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
        275                 280                 285

ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac          912
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
    290                 295                 300

acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac          960
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
305                 310                 315                 320

gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc         1008
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
                325                 330                 335

gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac         1056
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
            340                 345                 350

agc acg tac cgg gtg gtc agc gtc ctc acc gtc tgt cac cag gac tgg         1104
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His Gln Asp Trp
        355                 360                 365

ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca         1152
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
    370                 375                 380

gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gaa         1200
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
385                 390                 395                 400

cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag aac         1248
Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
                405                 410                 415
```

Sequence Listing

```
cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac atc        1296
Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
            420                 425                 430

gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc        1344
Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
        435                 440                 445

acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc aag        1392
Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
    450                 455                 460

ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca tgc        1440
Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
465                 470                 475                 480

tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc ctc        1488
Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
                485                 490                 495

tcc ctg tct ccg ggt aaa     tga                                       1509
Ser Leu Ser Pro Gly Lys
            500
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<210> 24
<211> 502
<212> PRT
<213> Homo sapiens

<400> 24

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Met Arg Thr Trp Pro Cys Thr Leu Leu Phe Phe Leu Leu Phe Ile Pro
  1               5                  10                  15

Val Phe Cys Lys Ala Met His Val Ala Gln Pro Ala Val Val Leu Ala
            20                  25                  30

Ser Ser Arg Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly
        35                  40                  45
```

Sequence Listing

Lys Ala Thr Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln
50 55 60

Val Thr Glu Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr
65 70 75 80

Phe Leu Asp Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val
85 90 95

Asn Leu Thr Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile
100 105 110

Cys Lys Val Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly
115 120 125

Asn Gly Thr Gln Ile Tyr Val Asn Asp Thr Glu Pro Cys Asn Asp Ser
130 135 140

Asp Asn Asn His Thr Ala Gln Pro Ala Val Val Leu Ala Ser Ser Arg
145 150 155 160

Gly Ile Ala Ser Phe Val Cys Glu Tyr Ala Ser Pro Gly Lys Ala Thr
165 170 175

Glu Val Arg Val Thr Val Leu Arg Gln Ala Asp Ser Gln Val Thr Glu
180 185 190

Val Cys Ala Ala Thr Tyr Met Met Gly Asn Glu Leu Thr Phe Leu Asp
195 200 205

Asp Ser Ile Cys Thr Gly Thr Ser Ser Gly Asn Gln Val Asn Leu Thr
210 215 220

Ile Gln Gly Leu Arg Ala Met Asp Thr Gly Leu Tyr Ile Cys Lys Val
225 230 235 240

Glu Leu Met Tyr Pro Pro Pro Tyr Tyr Leu Gly Ile Gly Asn Gly Thr
245 250 255

Gln Ile Tyr Val Ile Asp Pro Glu Pro Cys Pro Asp Ser Ala Glu Pro

Sequence Listing

260 265 270

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
275 280 285

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
290 295 300

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
305 310 315 320

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
325 330 335

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
340 345 350

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Cys His Gln Asp Trp
355 360 365

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
370 375 380

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
385 390 395 400

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
405 410 415

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
420 425 430

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
435 440 445

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
450 455 460

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
465 470 475 480

Sequence Listing

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
485 490 495

Ser Leu Ser Pro Gly Lys
500

<210> 25
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide TNFR1-EDF-EcoRI

<400> 25
ccggaattcc ggtctggcat gggcctctcc acc 33

<210> 26
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide TNFR1-EDR-IgGh

<400> 26
cacaagattt gggctctgct gtggtgcctg agtcctc 37

<210> 27
<211> 37
<212> DNA
<213> Artificial Sequence

<220>

Sequence Listing

<223> PCR primer, oligonucleotide IgG1-T1F

<400> 27
gaggactcag gcaccacagc agagcccaaa tcttgtg 37

<210> 28
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide IgG1-R-XbaI

<400> 28
gctctagagc tcatttaccc ggagacaggg agag 34

<210> 29
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide TNFR2-EDF-EcoRI

<400> 29
ccggaattcc gggcacccat ggcgcccgtc gcc 33

<210> 30
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide TNFR2-EDR-IgGh

Sequence Listing

<400> 30
cacaagattt gggctctgcg tcgccagtgc tcccttc 37

<210> 31
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide IgG-T2F

<400> 31
gaagggagca ctggcgacgc agagcccaaa tcttgtg 37

<210> 32
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide TNFR1-CF-BamHI

<400> 32
cgcggatccg ggaacatttc actggtcccc cacctag 37

<210> 33
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide TNFR1-NR-BamHI

Sequence Listing

<400> 33
cgcggatccg tcctcagtgc ccttaacatt ctcaatctg 39

<210> 34
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide TNFR2-CF-BamHI

<400> 34
cgcggatcca acgcaactac accctacgcc ccggag 36

<210> 35
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide TNFR2-NR-BamHI

<400> 35
cgcggatccg ctcccttcag ctgggggggct g 31

<210> 36
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide mgTNFR1-TNFR1-IgG-F

Sequence Listing

<400> 36
aaaagcaacg agaccaacaa gacctgccta cacaacgggt ccagggagaa gaacgatagt 60

gtg 63

<210> 37
<211> 62
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide mgTNFR1-TNFR1-IgG-R

<400> 37
ctccctggac ccgttgtgta ggcaggtctt gttggtctcg ttgctttttct tacagttact 60

ac 62

<210> 38
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide mgTNFR2-TNFR2-IgG-F

<400> 38
atggatgcaa actgcacgtc cccggagccc aacagcacat gccgg 45

<210> 39
<211> 42
<212> DNA
<213> Artificial Sequence

<220>

Sequence Listing

<223> PCR primer, oligonucleotide mgTNFR2-TNFR2-IgG-R

<400> 39
gcatgtgctg ttgggctccg gggacgtgca gtttgcatcc at 42

<210> 40
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide CD2F-EcoRI

<400> 40
ccggaattca tgagctttcc atgtaaattt gtagcc 36

<210> 41
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide CD2R-PstI

<400> 41
ctctgcagga cagctgacag gctcgacact 30

<210> 42
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide IgG-F-PstI

Sequence Listing

<400> 42
atctgcagag cccaaatctt gtgac 25

<210> 43
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide CTLA4F-EcoRI

<400> 43
ccggaattca tgaggacctg gccc 24

<210> 44
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide CTLA4R-PstI

<400> 44
ctctgcagaa tctgggcacg gttcaggatc 30

<210> 45
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide CD2-NT-F

Sequence Listing

<400> 45
taaagagatt acgaatgcc 19

<210> 46
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide CD2-CT-R

<400> 46
tgcaggacag ctgacagg 18

<210> 47
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide CTLA4-NT-F

<400> 47
ggataatcat gcacgtggcc cag 23

<210> 48
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide CTLA4-CT-R

Sequence Listing

<400> 48
tgcagaatct gggcacgg 18

<210> 49
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide mgCD2-CD2-IgG-F

<400> 49
cagtgtcgag aatgtcagct gtcctaaaaa tattacgaat gcc 43

<210> 50
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide mgCD2-CD2-IgG-R

<400> 50
ggcattcgta atatttttag gacagctgac attctcgaca ctg 43

<210> 51
<211> 64
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide mgCTLA4-CTLA4-IgG-F

<400> 51

Sequence Listing

atttatgtaa acgatacaga accgtgcaat gattcggata acaaccacac agcccagcct 60

gctg 64

<210> 52
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer, oligonucleotide mgCTLA4-CTLA4-IgG-R

<400> 52
aggctgggct gtgtggttgt tatccgaatc attgcacggt tctgtatcgt ttacataaat 60

ctg 63

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/01427

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07K 16/46

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 C07K 16/46, C07K 19/00, C12N 15

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Patents and applications for inventions since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search terms used)

Medline, Biosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,073,627 A (Immunex Corporation) 17 DECEMBER 1991 see the whole document	1
X, P ------ Y,P	EP1148065 A1 (ROSE-JOHN, STEFAN) 24 OCTOBER 2001 see column3, lines 20-40, claims	1 --- 2-5, 7-10, 12, 14, 15
Y	EP0464533 A1 (HOECHST AKTIENGESELLSCHAFT) 8 JANUARY 1992 see claims	2-5, 7-10, 12, 14, 15
Y	US 5861151 A (BRISTOL-MYERS SQUIBB CO.) 19 JANUARY 1999 see column7, lines 40-45, Fig,1	2-5, 7-10, 12, 14, 15
A	US 5349053 A (PROTEIN DESIGN LABS, INC) 20 SEPTEMBER 1994 see the whole document	1-35
A	US 5428130 A (GENENTECH, INC) 27 JUNE 1995 see the whole document	1-35
A	US 6165476 A (BETH ISRAEL DEACONESS MEDICAL) 26 DECEMBER 2000 see the whole document	1-35

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevence

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 DECEMBER 2002 (11.12.2002)	12 DECEMBER 2002 (12.12.2002)

Name and mailing address of the ISA/KR	Authorized officer
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Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR02/01427

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		JP 5503009 T2	27.03.93
		WO 9108298 A2	13.06.91
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		WO 9902711 A3	02.09.99